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**STUDIES ON INSECTICIDE RESISTANCE IN ANOPHELENE MOSQUITOES**

By

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ABSTRACT

The resistance spectra of different populations of a number of anopheline vectors of malaria were studied using some organochlorine, organophosphate, carbamate and pyrethroid insecticides. The species tested were Anopheles culicifacies from Sri Lanka, Pakistan and India, A. stephensi from India and Iran, A. sacharovi from Greece and Turkey, A. maculipennis and A. superpictus from Greece, A. hyrcanus from Turkey and A. albimanus from Panama and El Salvador. There was a diversity in the response to different insecticides both within species as well as between different species. With the exception of A. superpictus all other species showed either one or more populations with already developed multiple resistance or the potential for such development. Of significance may be the resistances shown by a number of these populations towards some OPs considered as potential alternatives to DDT in malaria control. Most of these variations were considered a result of the nature of the selection pressures exerted on the relevant populations. However, the differences observed in the response to malathion, in A. culicifacies and A. stephensi from that in the species A. sacharovi, A. maculipennis and A. hyrcanus, all from the Mediterranean region were attributed to a possible difference in the predominance of this resistance factor/factors. The malathion resistance in the former was common whereas it was rare in the latter group of species.

The high malathion resistance in a multiple resistant population of A. culicifacies from India was shown to be of an almost completely dominant nature. At least two genetic factors, one controlling the specific carboxyesterase mechanism and the other possibly a more generalised mechanism were suggested to be involved in this resistance, the latter in addition conferring cross resistance to fenitrothion. The resistance to this insecticide in the Iranian population of

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A. stephensi was of an incompletely dominant nature and a single gene involvement was suggested. The malathion selected (laboratory) Iranian population in which only the carboxyesterase mechanism could be demonstrated, showed no cross resistance to fenitrothion, and other OP's lacking the carboxyester bonds. Continued selection of the same population with fenitrothion, however, showed increasing trends in the tolerances to OP's, chlorphoxim, pirimiphos methyl and phoxim, suggesting a possible relationship between these resistances. In A. albimanus from El Salvador, evidence from use of synergists, only, had suggested possible involvement of carboxyesterases, and mfo's in the malathion resistance.

In A. sacharovi from Turkey, both fenitrothion and iodofenphos resistances were attributed to hydrolytic esterases as well as mixed function oxidases.

While the DDT resistance in A. culicifacies, and A. stephensi was attributed to the specific DDT-ase mechanism, that in the populations of A. sacharovi from Turkey was suggested to involve DDT-ase, mfo's and a third factor, possibly in the nature of a knockdown resistance mechanism. At least one or more of these mechanism were considered to have imparted cross resistance to the pyrethroid insecticides.

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### INTRODUCTION

Between the end of World War II and the present day residual insecticides have provided the main means of control of many of the principal insect-borne diseases of mankind. Their widespread use brought the inevitable consequence of resistance. Until the beginning of the present decade this was largely confined to the organo-chlorine compounds, except in a few instances such as in houseflies. Now trends in the development of resistance seen in terms of the number of species, types of insecticides, as well as geographical extent is presenting an ever increasing threat to the continued use of this means of control.

This situation is particularly serious in terms of mosquitoes and especially in anophelines, the transmitters of one of the world's commonest diseases, malaria. Recent years have shown a startling resurgence of the disease and resistance to the insecticides is considered a major contributing factor. Thus resistance resulting in control failures has necessitated the withholding of the use of organochlorine insecticides, in particular DDT, one of the most economical and effective insecticides used in many of the malaria control programmes in the past. In view of this many countries have implemented long term strategies involving the use of available alternatives such as organophosphates and carbamates for the control and hopefully eradication of this disease.

In many species of insects, the development of multiple resistance, has been attributed to a great extent to the selection pressure exerted by the extensive use of pesticides in agriculture. In recent years reports of malathion resistance have appeared in certain species of malaria vectors following the use of this insecticide in malaria control and this may be a forewarning to most malaria control

programmes now relying on this insecticide.

Some countries such as Sri Lanka, where malathion is now the main weapon against the vector Anopheles culicifacies, have advocated reserving the organophosphate insecticide, fenitrothion, as the next alternative to malathion, should resistance to the latter eventually develop. To this end, through collaboration with the agricultural authorities, the Anti Malaria Programme of the Ministry of Health, has curtailed the use of fenitrothion for agricultural purposes to avoid any early development of resistance to this insecticide.

Instances are known, however, where resistances to insecticides have developed to many compounds other than the selecting agent due to the phenomenon of cross-resistance. Such a situation has also been reported for the two insecticides malathion and fenitrothion.

The present study was primarily initiated with a view to gaining an insight into the nature of malathion resistance which has already developed in A. culicifacies in the Maharashtra State of India, this species being an important vector in most of the South East Asian countries. Apart from studying the nature of malathion resistance itself, it was also hoped to determine possible relationships between malathion resistance and that towards some potential alternative insecticides including fenitrothion.

In addition, attempts were made to establish the nature of the responses shown by different populations of a number of other anopheline vectors of malaria towards a range of insecticides of different groups.



## LITERATURE REVIEW

### Introduction

Since the first record of resistance to the organochlorine insecticide, DDT, in houseflies in 1946 (Wiesmann 1947) the problem of resistance has extended in terms of species of pests, their geographical extent, as well as the number and types of insecticides. Trends in the development of resistance have been documented in various reports, reviews and the global situation of the recorded cases are periodically updated by the World Health Organization. The present situation regarding the status of resistance in the anopheline mosquitoes, particularly, the vectors of malaria are reviewed in the W.H.O. technical report (1976) and by Georgiou and Taylor (1976).

### Detection of Resistance

In the early 1950's, the detection of resistance was a matter of conjecture based on observations of control failures. With the development of the W.H.O. standard technique, based on the principle of Busvine and Nash (1953), assessment of the changes in the susceptibility levels of populations were standardised. These trends were usually assessed either on a serial concentration, or serial time basis using the  $LC_{50}$  (lethal concentration for a 50% kill) or the  $LT_{50}$  (lethal time for a 50% kill) as the indices for comparison. The latter is utilised on the basis of an inverse relationship of time and concentration for an equitoxic effect (Pammel *et al.* 1964, Ariyaratnam and Brown 1969, Hamon and Sales 1970, Rongwariyam and Busvine 1973).

It has, however, been pointed out that measurement of resistance levels in terms of these values has limited significance in heterogeneous populations. According to Davidson (1958), Dyte and Blac'man (1967), Dyte (1970) and others, complete reliance on assessments based solely on these values could be misleading in that the true levels of resistances can be wrongly estimated, depending on the level of heterogeneity. In fact, the presence of resistant individuals may be missed altogether. Instead, Davidson (1960), Dyte (1970), Davidson and Zahar (1973) advocated the use of discriminating dosages, known to kill the susceptibles of a particular species. Of the many advantages of this technique the importance of early detection of resistance has been stressed particularly when the proportion of resistant individuals in a population is small. To confirm suspected cases of resistance continued testing of the offspring of the survivors of the discriminating dosage, to the same has been suggested.

#### Present Status of Resistance in Anopheline Mosquitoes

According to W.H.O. technical report (1976), and Georghiou and Taylor (1976), among the 42 cases of resistance recorded in 1975 in anopheline species, 24 were resistant to DDT, of which 21 showed double resistance, and 41 resisted dieldrin. Resistance to organophosphates, either to one or two, was reported in A. culicifacies from India, in A. sacharovi and A. hyrcanus from Turkey, in A. messeae from Romania, in A. sinensis from Ryukyn Islands and in A. albimanus from El Salvador. In addition A. sacharovi from Turkey and A. albimanus from El Salvador showed carbamate resistance.

### Development of Resistance in the Species Studied

#### A. sacharovi

A. sacharovi from Greece, was the first anopheline to develop insecticide resistance. Trends in the development of resistance in this species to DDT and dieldrin in various countries have been reviewed by various workers (Georgopoulos 1951, Hadjinicolaou 1954, 1957, Zulueta 1959, Brown and Pal 1971, Manouchehri et al., 1974b, Ramsdale 1975 and others).

The first indication of OP resistance in this species was observed in the Chukurova Plain of Turkey in 1974, where survivors to the discriminating dosages of fenitrothion, fenthion and propoxur were encountered (Ramsdale 1975). Further increases in resistance levels were shown by the spring of 1975. Here survivors were obtained with bromophos and malathion. Laboratory tests on the progeny of survivors, showed tolerance to propoxur, fenthion and fenitrothion. With malathion only the larval stages showed tolerance.

#### A. albimanus

A. albimanus from Central America showed multiple resistance, involving organochlorines, OP's and carbamates. (Details in section dealing with resistance spectra.)

#### A. culicifacies

The susceptibility tests made on this species from Sri Lanka, between 1961 to June 1973 with DDT have been summarised by Clarke et al. (1974). Since the first indication of resistance to this insecticide in early 1969, all subsequent tests had confirmed the presence of resistant individuals. In two localities continued re-testing had not shown significant increase in the levels of resistance, in spite of continuing spraying. The population had remained susceptible to all the other insecticides tested, namely dieldrin, fenitrothion, fenthion and propoxur (reports of the Anti-Malaria Campaign).

Rahman et al. (1959), Luen and Shalaby (1962), Shalaby (1968) Samson et al. (1974) have followed the development of DDT and dieldrin resistance in A. culicifacies in India.

DDT resistance in this species from Southern Iran has been reported by Zaini and Manouchehri (1973) and Manouchehri et al. (1975a). The latter workers claimed its susceptibility to both dieldrin and malathion.

Rajagopal (1977) reported the first indication of malathion resistance in this species from the Gujarat State of India. Compared to its susceptibility level in 1970 prior to the use of malathion in malaria control, the 1973 observations had revealed a fourfold increase in the value of the  $LC_{50}$ . The 100% mortality obtained at one hour exposure to 3.2% malathion in 1970 was reduced to 42.8% in 1973. Further, records of survivors to the discriminating dosages of malathion and fenitrothion were seen in the reports made available by the W.H.O.

#### A. stephensi

This species from Iran, first developed resistance to DDT in 1957 (Mofidi et al. 1958), and dieldrin in 1959 (Mofidi 1960). Manouchehri et al. (1975b) reported laboratory attempts to select for malathion resistance from the progeny of survivors of 3.2% malathion for one hour from two localities. The mortality with 5.0% malathion for one hour had decreased from 94% in the wild population to 46% in the selected one whereas the base line susceptibility data had earlier reported a 100% mortality at the same dosage (Manouchehri et al. 1966).

A population of this species from Bandar Abbas, Iran, was reported by Manouchehri et al. (1974a) to have remained susceptible to malathion in spite of 10 years of selection pressure. However, tests carried out in April 1975 in this area and in another, the Jiroft area,

reported the presence of individuals resistant to this insecticide in the former (Manouchehri 1976a). With 3.2% malathion, one and two hour exposures gave 55-64% and 93-95% respectively, while on 5.0% malathion the same exposures gave 74-80% and 98-99% respectively. The population from the Jiroft area which was subject to approximately 8 years of insecticide pressure, had continued to remain susceptible to this insecticide. 3.2% and 5.0% malathion for one hour exposures gave 91% and 100% mortalities respectively.

Manouchehri et al. (1976b) reported further increasing trends in the resistance level of the population from Bandar Abbas in the winter of 1976 where 3.2% malathion gave a 12-35% mortality at one hour exposure. 1.5, 2 and 4 hours exposures gave 49%, 82% and 100% respectively. The estimated  $LT_{50}$  was 90 minutes, compared with the 14 minutes with 3.2% malathion in the susceptible population (Manouchehri et al. 1975 b). This was a 6.4-fold increase in the resistance level.

#### Factors Influencing the Development of Resistance

A concise review of the factors determining the development of resistance in populations was made by Georgiou (1972). Keiding (1975 c,d) further elaborated some of these aspects based on information gathered during the sequential resistance development in the field populations of houseflies in Danish farms.

The presence of the genetic potential and sufficient selection pressure was indicated as essential requisites for resistance development in populations. Earlier assumptions of Wilson Jones and Davidson (1958) that resistant genes were rare in nature, were subsequently considered to be under-estimations, considering the rapid development of DDT resistance in houseflies and mosquitoes; and also the high frequency of dieldrin resistant genes observed in A. gambiae

(Service and Davidson 1964) and A. funestus (Service 1964) from unsprayed areas. Porter (1964) on the other hand considered the DDT and dieldrin resistant genes to exist in a low frequency in untreated populations, being maintained in a balanced polymorphism.

On the basis of differential responses shown by co-existing populations such as Musca domestica and Stomoxys calcitrans, presumed to have been subjected to comparable selection pressures, Keiding (1975c) suggests the possibility of variations in the genetic potential to develop different levels of resistance among species.

The process of co-adaptation suggested to be involved in resistance development, (Lerner 1958), was considered by McEneaney and Naegle (1968) to improve the competitiveness of the resistant genes and therefore in stabilising the resistance levels.

The dominance of resistant genes (Macdonald 1959, Georgiou 1969a, Flapp 1970, and Brown 1971) and their frequency, were shown to be important determinants of the speed of resistance development. In addition, the importance of population size, rate of its growth, relative isolation of populations and inbreeding were also cited. Brown and Pal (1971) have drawn attention to the importance of the stage of life at which selection is exerted. The speed of resistance development was also dependent on the proportion of the population and the number of generations subjected to selection pressure. The nature of the resistances involved was said to influence the effect of selection pressure exerted, in terms of its effect on mortality. Thus in D. melanogaster, King (1954, 1955) demonstrated faster development of DDT resistance at the lowest level of selection. In Tribolium castaneum Dyte and Blackman (1967) showed an initially high level of selection pressure to produce the lowest level of resistance, presuming that curtailment of reproduction had caused inbreeding of the strain. In

the case of dominant and monofactorial resistance (e.g. dieldrin resistance) or in instances of renewed selection of a reverted resistance (where co-adaption may not be considered essential) selections involving high mortality are considered to hasten the resistance development (Brown 1971).

Even in such instances, Rozeboom (1963) considers the selection of minor genetic factors to enhance the effect of major genes as was demonstrated in A. albimanus. Where more than one major genetic factor is involved, moderate pressure subsequently increased was considered to be most effective (Hoyer and Flapp 1968, Sawicki and Lord 1970). Georghiou (1972) considers this to simulate the nature of field selections where with the residual insecticides, partial selection of subsequent generations and the migrants are affected. Such selections could preserve both recessive and dominant factors, which may interact to increase the resistance levels.

Previous exposure to insecticides both related and unrelated to the selecting agent are claimed to enhance the speed of resistance development to the latter. According to Georghiou (1972) the speed of resistance development is initially slow in previously unselected populations when the major resistant factors are increasing and the co-adaptation process in progress, after which the process is accelerated. The importance of types of insecticides used, nature of resistance mechanisms involved, the sequence and combinations used have all been discussed by Keiding (1975c).

#### Resistance Mechanisms

Refined toxicological, biochemical, physiological and genetic techniques, along with the use of synergists, have facilitated the gaining



of insight into some of the mechanisms of resistance. Some of the recent reviews on this aspect have been made by Busvine (1971a, 1973), Oppenoorth (1971), Georgiou (1972), Sawicki (1971 a, b, 1975a), Flapp (1976), and Oppenoorth and Welling (1976).

Factors influencing the fate of the insecticide within an animal are broadly categorised by Winteringham (1969). On a theoretical basis, any changes affecting penetration, distribution and target site interaction of an insecticide are claimed to contribute to resistance (Chadwick 1955, Winteringham 1969). Of those changes so far established, reduced penetration, increased detoxication, and altered site of action have been cited as of prime importance (Oppenoorth and Welling 1976).

#### Reduced penetration

This mechanism can influence an already existing detoxication mechanism by increasing the time available for its action. While several authors have related reduced insecticidal penetration with resistance in houseflies, Flapp and Hoyer (1968b) have reviewed reports pertaining to this aspect in a number of insects. A gene pen (Sawicki and Farnham 1967, 1968a) and a gene tin (Hoyer and Flapp 1968) were reported to control reduced penetration of insecticides. Sawicki (1970) showed these to be allelic. Hoyer and Flapp (1968) and Flapp and Hoyer (1968b) showed this gene to impart low levels of resistance to most insecticides while Sawicki and Lord (1970) demonstrated this to be most effective on non polar compounds. Sawicki and Farnham showed the expression of this gene to be intermediate in delaying penetration but recessive to kill (Sawicki and Lord 1970). However it is known to augment the effect of certain other resistance factors. Hoyer and Flapp (1968), Flapp and Hoyer (1968b), Sawicki and Farnham (1968a), Sawicki (1970) and Georgiou (1971) have shown the mechanism to be non-specific and selected by a variety of insecticides in houseflies.



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### Increased metabolism

On the basis of current knowledge, the mechanisms of detoxication are broadly categorised as DDT dehydrochlorinase, hydrolases, glutathione-transferases and mixed function oxidases.

### DDT-dehydrochlorinase

Studies on DDT-ase have been primarily confined to the houseflies and reviewed by Lipke and Kearns (1960), Perry (1964), O'Brien (1967) and Oppenoorth and Welling (1976). A gene Deh or DDT-ase (Oppenoorth 1965a) reported to control the activity of this enzyme (Tsukamoto and Suzuki 1964, Oppenoorth 1965a, b). Either different or closely linked alleles producing multiple forms of this enzyme, which showed different substrate specificity were suggested to produce varying resistance spectra (Oppenoorth 1965a). In addition both qualitative and quantitative differences among the susceptible and the resistant strains have been indicated. Grigolo and Oppenoorth (1966) have suggested the possibility of a mutational step from alleles of low to intermediate activity in the susceptible, to those with high activity in the resistant ones.

While the synergistic action of DMC on DDT through inhibition of DDT-ase activity was demonstrated by Perry et al. (1953), Moorefield and Kearns (1955), Cohen and Tahori (1957) and Oppenoorth 1965b) others have demonstrated a similar inhibition of this enzyme by the more stable F-DMC.

Considering the differences encountered in the enzymes within various strains of houseflies, similar variations in other species have been suggested by Oppenoorth and Welling (1976). Thus Pillai et al. (1963) showed a DDT-ase activity in the American strain of A. aegypti to exhibit a substrate specificity differing from that in the Asiatic strain of the same species, as well as from that in houseflies. Variations were also reported in Culex species by Kimura et al. (1965).

### Mixed function oxidases (mfo's)

The role of mfo's in resistance in insects was first elucidated through the action of synergists known to inhibit these enzymes (see reviews by Wilkinson 1968 and by Casida 1970). The specific action of piperonyl butoxide (PB) and sesamex as inhibitors of this enzyme system, was suggested by Hodgson and Casida (1960) and demonstrated in insects by Sun and Johnson (1960). The synergistic effect of sesamex (Elderfrawi *et al.* 1960), and PB (Georghiou and Metcalf 1961) were demonstrated with carbamate insecticides. The association of mfo's with resistance was also demonstrated by Schonbrod *et al.* (1965), Tsukamoto and Casida (1967 a, b) and Khan *et al.* (1970).

Oppenoorth and Welling (1976) has updated and reviewed information correlating mfo activity with resistance to varying groups of insecticides i.e. organochlorines, organophosphates, carbamates and pyrethroids.

A gene DDT-md (Oppenoorth and Hour 1968) and Ses (Sawicki and Farnham 1967) was found to control mfo activity involved in the DDT degradation in a OP selected housefly strain. Either the same gene or one closely associated with it was demonstrated to control oxidative mechanisms involved in the metabolism of DDT, methoxychlor and certain OP's (Sawicki and Farnham 1968). However the resistance conferred by this alone was of a weak to moderate nature. Oppenoorth (1972) showed that its expression could be modified by interaction with other genes such as those of delayed penetration, DDT-ase, OP detoxifying enzymes. Sawicki and Farnham (1968) showed it to be intermediately dominant in nature and that its effect could be suppressed by sesamex. Oppenoorth (1967) and Sawicki (1972) have further demonstrated two additional genes also controlling mfo activity in houseflies, one of which conferred weak to strong resistance to varying OP's. This was sesamex suppressible. The other, was considered to be possibly involved in oxidative

dealkylation (Oppenoorth 1972). Georghiou (1971) also isolated two factors on chromosome 2 in houseflies conferring weak to moderate resistance to some OP's and carbamates and a third (on chromosome 5) which was weak in its expression.

Carbamate resistance has been attributed mainly to oxidative mechanisms. In Culex pipiens fatigans, the resistance to propoxur was caused by the mfo system (Shrivastava et al. 1970) with the penetration reducing factor enhancing its effect. Yamamoto and Casida (1966) showed the combined effect of mfo's as well as a Kdr (Knock down) mechanism to produce resistance to pyrethroids.

#### Hydrolases

Both carboxyesterases and phosphatases fall within the category of hydrolases. Literature pertaining to the former is reviewed under the section dealing with malathion resistance.

#### Phosphatases

The presence of a certain level of phosphatase activity in resistant strains of houseflies was demonstrated by Oppenoorth and Van Asperen (1960, 1961), Oppenoorth and Voerman (1975), Welling et al. (1971), Lewis and Sawicki (1971). Metabolites presumed to be products of these enzymes were demonstrated.

In view of the reduced vulnerability of the phosphorothioates to phosphatase attack, due to the electrophilic phosphorous atom, the possibilities of these compounds being metabolised by these enzymes to any considerable extent is doubted by Oppenoorth and Welling (1976). Further, the importance of this metabolic pathway appears to be controversial, in spite of such assumptions being made by Matsumura and Hogendijk (1964 b) and others. In view of the same byproducts being produced through the action of different enzyme systems such as oxidative, GSH transferases, and phosphatases, Oppenoorth and Welling (1976) have indicated the possible misinterpretations that could occur in attempting

deductions as to the nature of metabolic pathways, purely based on the structure of the metabolites formed. He has drawn attention to the fact, that in instances where this enzyme activity was postulated, other resistance mechanisms (reduced penetration, or oxidases) had also been in existence.

#### Glutathione-transferase (GSE)

The significance of this enzyme in OP resistance has not yet been fully established. Apart from the study of metabolites producing inconclusive evidence, lack of appropriate synergists has failed to establish its exact function.

A gene controlling this enzyme was identified by Lewis (1969), Lewis and Sawicki (1971) and Oppennoorth et al. (1972). In addition, 2 additional genes, one of which was associated with carboxyesterases, was also reported. Lewis and Sawicki (1971) demonstrated its activity in a multiresistant strain of houseflies, and Oppennoorth et al. (1972) and Devnnashire (1973) showed its effect on parathion in a malathion resistant strain. Its role in resistance was also reported in houseflies by Motoyama and Dauterman (1972).

#### Altered site of action

##### Altered cholinesterase

Reports of OP and carbamate resistance presumed to be caused by a reduced sensitivity of the target site, acetylcholinesterase (AChE) are increasing. The first clear evidence of the involvement of this mechanism in resistance was made in the OP resistant spider mite Tetranychus urticae by Smissaert (1964) and later by others in other species. Detailed studies of this mechanism have demonstrated the existence of strains with different degrees of resistance as well as varying levels of OP insensitive AChE's.

Elderfrawi et al. (1970), Nolan et al. (1972), Tripathi and O'Brien (1973a) and others have indicated AChE in houseflies and cattle-

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ticks to be apparently composed of at least 4 isozymic forms which differ in their sensitivity to inhibition by OP<sup>s</sup>. The resistance was therefore attributed to either a change in the relative amounts of the different forms or a change at the active site of some of the isozymes. Devonshire (1975) showed evidence of these isozymes being interconvertible and therefore probably representing different stages of aggregation. Tripathi and O'Brien (1973b) showed the resistance to Rabon (tetrachlorvinfos) in a strain of houseflies to be caused by this mechanism. They concluded that there is a possible change in enzyme structure which affects the binding sites of insecticides and does not involve the site affecting AChE. Sawicki (1975a) has indicated that the resistance conferred by this mechanism to be low in houseflies.

Ayad and Georgiou (1975) on the other hand attributed the high degree of resistance shown towards propoxur, parathion, several other OPs and carbamates in a strain of A. albimanus to be caused by a sharp reduction in the sensitivity of AChE. Georgiou et al. (1974) showed this mechanism to be due to a single gene allele, and presumes it to have evolved as a response to extremely severe selection pressure exerted by the widescale use of agricultural insecticides. Considering the significance of this defence mechanism Georgiou and Taylor (1976) suggest that the selection pressure has "enabled the efficient AChE resistance mechanism to predominate by overpowering the usual metabolic defence mechanisms". They consider this a forewarning of a possible direction of evolution of OP and carbamate resistance where selection pressure exceeds the capacity of the metabolic defences in a population. Oppenoorth and Welling (1976) on the other hand are of the opinion that the mechanism could exert its effect only if at least some amount of detoxication enzymes are in existence.



#### Gene for knockdown resistance (Kdr)

This gene is known to have an effect both on knockdown as well as on mortality. The three genes, kdr, (Milani and Travaglini 1957), kdr-O (Milani and Franco 1959), and kdr-NPR (Farnham 1971) were shown to be allelic by Farnham (1972).

A cross-resistance between DDT and pyrethrins was shown by Busvine (1951) in a strain of houseflies. Farnham (1971, 1973) showed the importance of kdr-NPR gene on resistance to both natural and synthetic pyrethroids. In view of the cross resistance shown to those compounds attacking nerve and sensory cells, it has been postulated that kdr may either alter the access of insecticide to the site of action, or alter the site of action itself. Milani and Travaglio (1957) showed the effect of the kdr gene to be affected by modifier genes. Grigolo and Oppenoorth (1966) showed a DDT-ase of low activity to enhance the effect of this gene. Keiding (1963) has suggested a greater abundance of the gene kdr in Europe based on studies in houseflies. A type of resistance comparable to the above was also reported for Culex tarsalis (Plapp and Hoyer 1968a).

#### Cyclodiene and lindane resistance

Evidence suggesting the importance of altered site of action in cyclodiene and lindane resistance is based on negative evidence (Oppenoorth and Welling 1976).

#### Cross Resistance, Multiple Resistance and Multiplicate Resistance

Busvine (1968), Sawicki (1975c) and Oppenoorth and Welling (1976) defined cross resistance as that conferred by a single defence mechanism towards a group of compounds, which are usually the selecting agent as well as those related to it, in a strain of insects. In multiple resistance 2 or more independent defence mechanisms existing together each impart resistance (cross-resistance) to a different group



of insecticides. Sawicki (1975b) has pointed out that the nature of this type of resistance can be variable and could develop in several stages as a consequence of either simultaneous or the consecutive use of several insecticides. The question of distinguishing between cross and multiple resistances in instances where linkage of genetic factors may exist as cross resistance, is discussed by Oppenoorth and Welling (1976). Here an insecticide selecting a rare mutant could simultaneously select the gene allele linked with it. If another resistant gene is also in existence in the population, the nature of the expression of the resistances will depend on the type of combination of the relevant alleles. Busvine (1971 b), has described multiplicate resistance in a strain of insects as that resulting when two or more mechanisms co-exist in the same organism and impart resistance towards the same insecticide. He and Sawicki (1975 c) indicated the importance of studying resistance spectra, particularly in cross and multiple resistances in attempts to identify the relevant resistance mechanisms. However, Sawicki (1975 c) has drawn attention to the fact that possible interactions of certain resistant mechanisms could mask the characteristics of resistance spectra and therefore not allow the proper identity of the mechanisms involved. The variations in the degrees of resistance, and the diversity of resistance spectra observed among many species, particularly towards the OP compounds, have been attributed to such interactions by Winteringham and Hewlett (1964).

Interactions between different resistance mechanisms have been studied to some extent through a combination of toxicological, biochemical, physiological and genetic techniques (Georghiou 1971, Sawicki 1970, 1973 a, Farnham 1973). Almost all studies made so far have been limited to houseflies, for various reasons, the ease of maintenance being an important factor. In these studies, multiplicate resistances have been resolved into their components, and the individual mechanisms studied. Then, by the sequential recombination of the components, the effect of

their interactions have been established. Sawicki (1975 c) has reviewed some of these interactions between various mechanisms of resistance to insecticides in insects.

Thus in a strain of houseflies while each gene in isolation conferred only a limited degree of resistance to a particular compound sequential recombination of these re-established the initial resistance level. The study showed the types and extent of interactions between the resistant genes. Enhancement of these factors was most effective between genes controlling detoxication mechanisms and reduced penetration. The study made by Sawicki (1973 a) on the individual effects of the 3 different combinations of the delayed penetration mechanism, the *mfo*: and the DDT resistance showed the production of different cross resistance spectra. This demonstrated the complexities involved through the different ways by which various resistant factors could influence each other and account for the great variety of cross resistance spectra seen in different strains of houseflies. Very high resistances to many OP: in strains with polyfactorial inheritance was considered to be a result of such interactions.

The penetration delaying mechanism has so far been shown to be a major interactant, particularly with detoxifying mechanisms. This was found to augment the resistances towards parathion (Flapp and Hoyer 1968b), DDT, dieldrin, and various OP: (Hoyer and Flapp 1971). Further, its action was shown to depend to a great extent on the nature of the resistance mechanism with which it interacts. In a malathion resistant strain, it enhanced the DDT resistance due to gene Ddh 24 times, but the malathion resistance conferred by an esterase was increased only 3 times. There was no increase in diazinon resistance controlled by a *mfo*. Sawicki and Lord (1970) observed its enhancing effect on detoxifying enzymes involved in demethylation. Farnham (1974) showed no effect on a DDT resistance resulting from a non-metabolic

process controlled by the gene kdr. Although a possible correlation between reduced penetration and malathion resistance (Matsumura and Brown 1963) and that of DDT resistance (Pillai and Brown 1965) has been suggested in Aedes aegypti, the probable involvement of other genes has also been considered in such instances. In C. tarsalis the resistance to parathion has been attributed to the interaction between delayed penetration and increased metabolism (Apperson and Georgiou 1975b). Similarly, interaction of this factor with increased metabolism was considered to cause high carbamate and OP resistance in the propoxur selected population of A. albimanus (Ariaratnam and Georgiou 1975). The DDT resistance in a strain of houseflies caused by an mfo mechanism (DDT-md) was enhanced only 6 times by Pen (Sawicki 1973a) but that attributable to DDT-ase increased 30 times (Hoyer and Flapp 1971). Although Farnham (1974) demonstrated that delayed penetration did not enhance the resistance to DDT by the gene kdr Grigolo and Oppenoorth (1966) showed an intermediate DDT-ase which had no significant resistance of its own, to act as a dominance modifier on the gene kdr, whereby the recessive kdr was converted to a dominant gene. The resistance to DDT was therefore greatly enhanced. Devonshire and Sawicki (1974) demonstrated a similar situation where the resistance of a less sensitive AChE towards OPs, was increased by a sesamex suppressible detoxifying factor.

Attention has been drawn by Sawicki (1975 c) to the absence of sufficient evidence as yet to establish interactions between detoxifying mechanisms themselves.

Interactions have also been shown between the synergists piperonyl butoxide and sesamex with diazinon by Sawicki and Farnham (1968a) who also demonstrated the opposing effects of the methylenedioxypheyl compounds on the two different mechanisms of resistance, the hydrolytic GSH dependant and oxidative mechanisms. Sesamex was shown to antagonise the former mechanism while with the oxidative

mechanism there was synergism. Welling et al. (1974) pointed out that although SV<sub>1</sub> inhibits the oxidative metabolism of malaoxon, no effect on malathion should be anticipated since the latter on its own is also known to inhibit the oxidative degradation of malaoxon. However, since the oxidative product of SV<sub>1</sub> inhibits the carboxyesterase degradation of malathion SV<sub>1</sub> is a synergist for malathion. This further demonstrated the complex interactions involved in multiplicate resistance.

Collaborative studies by Keiding (reviews 1974 a, 1975 a, b, 1976, etc.) and Sawicki (1974 a, 1975 b) relating genetic evidence with the history of sequential resistance in houseflies in Danish farms, have clearly demonstrated the effects of selections with insecticides on imparting cross resistance to others, (including those not used for control) depending on the mechanisms selected. Correlating the field observations with the resistance mechanisms, at different stages of selection, information regarding sequential resistance development has been obtained to some extent. Both Sawicki (1970) and Georghiou (1971) have suggested possibilities of deducing the approximate sequence in which the resistant genes would have been selected in houseflies. Sawicki (1975 b) further made attempts to speculate on the long term effects of selection with insecticides in terms of its effect on alternative compounds used subsequently. The long term effect of the selection of DDT resistance on resistance to diazinon, and pyrethroid compounds which were subsequently used have been discussed Sawicki (1975 b).

Keiding (1967) in his detailed discussion on the sequential resistance development in houseflies has drawn attention to the fact that resistant mechanisms quite unrelated to selecting agents can also be selected. Therefore Sawicki (1975 b) points out the limitations of attempting to establish causes of resistance on a basis of history of selection or even of making suggestions of suitable alternatives,

irrespective of information on the nature of mechanisms involved.

The development of multiple resistance in houseflies in California has been described by Georghiou (1971). Georghiou and Hawley (1971) demonstrated that, in addition to the retention of malathion resistance, resistance towards both diazinon and ronnel had increased in spite of the insecticides being replaced by alternatives. Georghiou (1972) suggests that resistance mechanisms present in a population may continue to be selected by the alternatives resulting in multiple resistance. In the case of dieldrin, however, the resistance level was not affected, possibly due to the resistance mechanism being independent of carbamate selection pressure (Georghiou and Metcalf 1963).

Multiple resistance in A. albimanus in Central America resulting from the sequential use of numerous insecticides is discussed under Resistance Spectra.

Possibilities of variations in the geographical distribution of certain resistance genes were suggested by Sawicki (1975 a). This was exemplified by the absence of records of sesamex suppressible OP resistance in American strains of houseflies. He suggested that most of the DDT resistance in the USA was due to DDT-ase, while that in Denmark was attributed to kdr and possibly DDT-md. Further, malathion resistance in the USA was known to be primarily due to carboxyesterases, while this was less common in Denmark. He has suggested that such information should be made use of in the long-term sequential use of insecticides. The importance of identification of resistant genes such as that of AChE causing resistance to most compounds has been stressed. In addition, the necessity of establishing the insecticides selecting such genes, and those selecting resistance to unrelated compounds has been pointed out, so as to minimise their use.

### Resistance Spectra

The importance of studies on resistant spectra in the identification of the resistance mechanisms has been suggested by many workers.

Davidson's studies on cross resistance of DDT resistant populations of A. sundaicus (1957), A. albimanus (1963 a) A. quadrimaculatus (1963 b), and those of Davidson and Jackson on A. stephensi (1961) were considered by Busvine (1968) to be insufficient data for identification of the resistance mechanisms involved. A typical dieldrin type of resistance spectrum was demonstrated by Davidson in A. gambiae (1956) and in A. albimanus (1963 a). Response to DDT analogues was studied by Kimura et Brown (1964) in C. fatigans. Perry (1966) and Pillai et al. (1963) in Aedes aegypti and Plapp et al. (1965) in Culex tarsalis. With the exception of A. aegypti, involvement of other resistance factors in addition to DDT-ase have been suggested. This has been substantiated by the failure to completely eliminate resistance with synergist DMC in C. tarsalis.

Rongariyam and Busvine (1975) studied the cross resistance patterns, and the effects of synergists on 5 species of mosquitoes: Aedes aegypti, Culex pipiens fatigans, A. stephensi, A. gambiae and A. quadrimaculatus. In C. p. fatigans, A. stephensi and A. quadrimaculatus, DDT-ase involvement was indicated, while in others the nature of the cross-resistance pattern (which involved pyrethroids also) and synergism by PB suggested the involvement of mfo's.

Prasittusak and Busvine (1977) compared the response of 8 strains of DDT resistant mosquitoes to pyrethroids. All except one strain showed low levels of permethrin resistance. Two strains, one with only high DDT, and the other with both DDT and permethrin resistance, were tested with methoxychlor and synergists. Part of the resistance was attributed to DDT-ase. However, cross-resistance in both strains to 3 other pyrethroids suggested association with an unknown mechanism,

the effect of which was considered to be augmented in the strain having the high permethrin resistance. The latter in addition also had an mfo involvement. Apperson and Georghiou (1975a) studied the changes in the resistance spectrum resulting from selection with methyl parathion, in a strain of Culex tarsalis. Considering the broad spectrum and the high level of resistance observed, involvement of more than one mechanism was suggested. Georghiou (1975a) based on the observations on the effects of selection above pointed out that when the genetic potential is in existence, selection with alternative insecticides could accelerate the speed of resistance development. Apart from the high level of resistance imparted to the selecting agent and its relatives, selection could also confer lower levels of cross-resistance to other compounds. Apperson and Georghiou (1975b) showed the parathion resistance in this OP resistant strain to be caused by a reduced penetration of insecticide as well as its increased detoxication. Strong synergism with DEF and not with FB, had implied involvement of hydrolytic in contrast to oxidative detoxications. The OP resistances were shown genetically to involve more than one resistant factor (Apperson and Georghiou 1975 c). Suzuki (1968) showed a multiple resistance involving cross-resistance to fenthion, fenitrothion, chlorpyrifos and malathion following selection of C.p. pallens with Abate.

The multiresistance in Culex p. quinquefasciatus in California was correlated with the extensive usage of agricultural insecticides in addition to direct larvicidal pressure (Georghiou 1975b). The populations showed a broad OP resistance, which extended even to those compounds not used for either agriculture or for mosquito control which was therefore attributed to cross-resistance. Strong synergism with DEF suggested the involvement of hydrolytic esterases. Stone and Brown (1969) and Stone (1969) had shown hydrolytic enzymes to be of prime importance in fenthion metabolism in a strain of the same species from Burma.



In the propoxur selected strain of this species on the other hand, the high rate of metabolism of propoxur, carbaryl and other carbamates were attributed to mfo enzymes (Shrivastava *et al.* 1970, 1971). The resistance studies made on another strain of C.p. quinquefasciatus with 12 experimental or new compounds including OPs, carbamates, pyrethroids and insect growth inhibitors showed high resistance towards chlorphoxim, cyanox and OMS 1342. The strain was resistant to DDT, susceptible to biodegradable analogues of DDT, and to pyrethroids. Ranasinghe and Georghiou (1976) attempted isolating two OP resistance mechanisms, an esterase, and an MFO-mediated mechanism by using synergists PB and DEF in a multiresistant population of C.p. quinquefasciatus.

In A. albimanus the first indication of resistance to malathion, accompanied by cross-resistance was reported from El Salvador in 1970 (Breeland et al.). Here an indirect selection with agricultural insecticides was demonstrated by correlating resistance in the wild population with the aerial spraying of malathion. An organochlorine resistant population from El Salvador exposed in the field to a variety of insecticides and showing tolerance to propoxur, carbaryl, malathion, fenitrothion and others was further selected with propoxur in the laboratory (Ariaratnam and Georghiou 1971). The resistance spectrum of the selected larvae extended to include carbaryl and several OPs, showing an enhancement of the low level of OP resistance which existed in the field. The involvement of a variety of detoxication enzymes for this multiple resistance were indicated by the use of synergists. Thus, while carboxylesterases were suggested as conferring malathion resistance, carbamate resistance was attributed to an mfo system.

The strong antagonism of PB with the phosphorothioate compounds indicated inhibition of their activation step. They attributed the lack of synergism of the phosphates to an attack on the thionate prior to its conversion to the former and/or to a nonoxidative degradation process. The low levels of synergism of paraoxon with DEF had



indicated a demethylation mechanism. The failure to synergise a portion of the high carbaryl and propoxur resistance in the population with any of the known synergists had suggested a reduced penetration factor. This was confirmed later by Ariaratnam and Georgiou (1975) who demonstrated an interaction of metabolism and reduced penetration in carbaryl resistance. However, presence of an additional factor, the effect of which was considered to be enhanced by the other two mechanisms was claimed to be responsible for the very high resistance. A strain was subsequently selected by Ayad and Georgiou (1975), in which the carbamate and OP resistance was not dependent on either metabolism, or reduced penetration, but was attributed to a reduction in the sensitivity of AChE. The effect of this was extensive, encompassing all the carbamates and a variety of OPs tested. The same genetic factor was claimed to cause resistance to propoxur, carbaryl, landrin, parathion and methyl parathion. In addition, the resistance to all OPs and carbamates was shown to be strongly genetically dominant.

Davidson and Sawyer (1975) also studied a population of A. albimanus from El Salvador, and showed it to resist DDT, OMS 1476, HCH, dieldrin, malathion, fenitrothion, dursban, parathion and propoxur. Towards fenitrothion, only the larval stages showed an increased tolerance. In addition, a monofactorial mode of inheritance of propoxur resistance was indicated which was shown to segregate independently from DDT and dieldrin resistances.

A great deal of work has been done on the resistance spectra of houseflies. Bell (1968) studied the response of 4 resistant strains of Musca domestica to a wide range of compounds in an attempt to deduce the possible resistance mechanisms involved. He demonstrated the varying cross resistance patterns caused by different resistance mechanisms. Having used a wider range of compounds, he was able to

demonstrate presence of additional resistance mechanisms in the same strains than had been reported by earlier workers. He has also pointed out the difficulties in attempting to identify mechanisms and postulate the number of genes involved in resistance, either biochemically or through study of resistance spectra.

#### Resistance to malathion

As most of this thesis concerns resistance to malathion this subject is reviewed separately.

O'Brien (1960) has indicated the pathways of malathion metabolism, while Oppenoorth and Welling (1976) has shown the theoretically possible primary sites of attack on malaoxon, the toxic analogue of malathion (Fig. 1). The metabolic process involved in activation of (oxidation or desulfuration) the organophosphate insecticides to the antiesterases has been reviewed by Metcalf (1969).

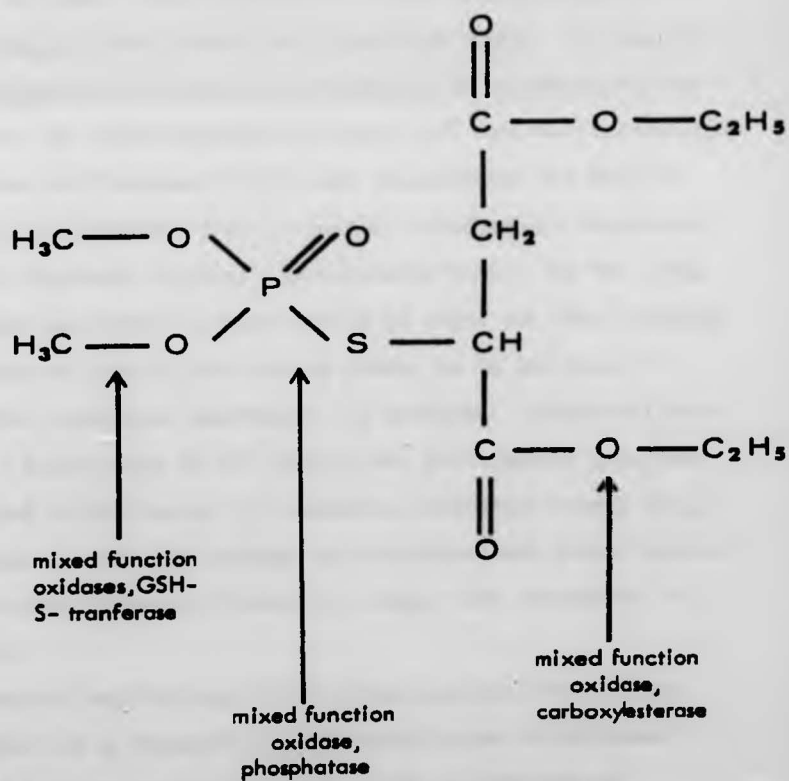
Biochemical and genetic studies, in combination with those involving the use of synergists and the determination of resistance spectra, have been utilised in attempts to elucidate the nature of malathion resistance in insects.

The commonest organophosphate resistance factor is considered to be a gene controlling the modified carboxyesterase. According to the mutant aliesterase theory of Oppenoorth and Van Asperen (1960) the carboxyesterases and phosphatases are mutant alleles of the aliesterases (carboxyesterases) of the susceptible forms. The involvement of carboxyesterases in resistance dates back to the study of van Asperen and Oppenoorth (1959). This was further demonstrated in various insect species by O'Brien (1960), Matsumura and Brown (1961, 1963), Dauterman and Matsumura (1962), Bigley and Flapp (1962), Matsumura and Hogendijk (1964a), Dyte and Rowlands (1968), Townsend and Busvine (1969), Welling and Blackmeier (1971) and others. Resistance to malathion as a

Fig.1

Theoretically possible sites of attack on  
malaoxon.

( Oppenoorth and Welling, 1976 )



result of hydrolysis at the carboxy<sup>1</sup>ester bond, resulting in the monoacid formation, was suggested by Darrow and Flapp (1960), Matsumura and Brown (1961, 1963) and Bigley and Flapp (1962). Busvine and Townsend (1969) and Welling and Blackmeier (1971) also established the importance of carboxy<sup>1</sup>esterase in resistance on the basis of the metabolites formed.

The specificity of carboxy<sup>1</sup>esterase action only on compounds containing the carboxy<sup>1</sup>ester bonds, such as malaoxon, malathion, phenthoate, acethion etc. was demonstrated in Chrysomya putoria by Busvine et al. (1963). Further, a highly malathion resistant strain of houseflies did not show cross resistance to other organophosphate compounds (Fay et al. 1958, Schoof and Kilpatrick 1958). A strain of Tribolium castaneum with the specific resistance to malathion and its analogues, showed no cross-resistance to other OP<sup>i</sup> such as fenitrothion and others. Dyte and Blackman (1972) also demonstrated the lack of cross resistance to compounds such as phoxim, iodofanphos, dimethoate and others, all compounds without carboxy<sup>1</sup>ester bonds. On the other hand, a tolerance was shown to those having at least one ethyl carboxy<sup>1</sup>ester bond moiety as part of the leaving group, as in the case of malaoxon, diethyl malathion, phenthoate and acethion. Absence of cross resistance to a large range of OP<sup>i</sup> lacking the carboxy<sup>1</sup>ester group was also demonstrated in the larvae of a malathion resistant strain of C. tarsalis, whereas a sevenfold increase in resistance was shown towards malaoxon and diethyl analogues (Darrow and Flapp 1960, Dauterman and Matsumura 1962).

Oppenoorth and Welling (1976) having analysed the studies made on the effect of a number of malathion analogues on different species by many workers, have suggested that the specificity of carboxy<sup>1</sup>esterases in resistant strains is more related to the size of the alkoxy groups than to the carboxy<sup>1</sup>ester groups of the insecticides concerned.

Synergism of carboxylesterases by the inhibitor of this enzyme triphenyl phosphate (TPP) has been demonstrated by Plapp et al. (1963), Dyte and Rowlands (1968), Townsend and Busvine (1969) and Welling et al. (1974). TPP is used as a tool in the laboratory diagnosis of the malathion specific resistance mechanism.

The highly specific malathion resistance in strains of houseflies was shown by Oppenoorth (1959) to be monofactorial in its inheritance. Malathion resistance in Culex tarsalis was attributed by Matsumura and Brown (1961), Plapp et al. (1961) to a single incompletely dominant genetic factor. However, here, although the  $F_1$  and  $F_2$  generations showed results in agreement with the monofactorial hypothesis of inheritance, the backcross progeny had shown a 3:1 segregation. This they attributed to additional genes or modifiers. It is therefore likely that more than one resistance mechanism was involved, genetic evidence alone being insufficient to establish the number of resistance factors involved. Malathion resistance in Culex tarsalis has been reviewed by Oppenoorth (1965 c).

In Aedes aegypti, the low malathion resistance was not attributed to either carboxylesterases or phosphatases (Matsumura and Brown 1961, 1963), but a decreased penetration of the insecticide in the larvae was indicated. The resistance was shown to be polygenic.

In view of reports of cases of OP resistances without associated low aliesterase activity (Oppenoorth 1959, 1967) possible existence of other genetic mechanisms has been suggested. Possibilities of malathion resistance due to phosphatases in addition to carboxylesterases were suggested (Matsumura and Hogendijk 1964a, and Main and Dauterman 1967, and others). This was on the basis that the quantities presumed to be carboxyester products alone, were insufficient to account for the total amount of metabolites formed. Welling and Blackmeier (1971), Welling

et al. (1974), however, consider the necessity for further evidence for establishing the importance of this metabolic pathway.

Nakatsugawa and Dahm (1968), Motoyama and Dauterman (1974), and Welling et al. (1974) have demonstrated the production of the same metabolites by different enzyme systems including those of oxidative and hydrolytic degradations. Therefore possibilities of misinterpretations of the nature of detoxication enzymes based solely on the metabolites formed were pointed out by Welling et al. (1974) and Sawicki (1975).

In contrast to the malathion specific mechanism discussed earlier, Champ et al. (1970 b), Dyte and Rowlands (1970), Dyte and Blackman (1971), reported a malathion resistant strain of Tribolium castaneum showing a broad spectrum of resistance to a wide range of OP compounds. This included fenitrothion, diazinon, phenthoate and others. In addition, there was tolerance towards the carbamates, carbaryl and arprocarb (propoxur) and to the organochlorine lindane. It was not known, if the latter was due to cross resistance or multiple resistance. TFP did not synergise malathion resistance, showing absence of the specific mechanism. The main metabolites shown by biochemical studies were suggestive of mfo involvements. The failure to demonstrate synergism with the appropriate synergists was suggested to be a result of a rapid breakdown of the latter. A third strain of this species was also found in which TFP could synergise only some of the resistance, thereby indicating two mechanisms conferring malathion resistance, one of these being non-specific. A similar type of resistance was shown in houseflies by Sawicki and Farnham 1968 and Sawicki (1970, 1973a), and in which tolerance towards malaoxon was higher than that towards malathion. Synergism shown by sesamex in these insecticides demonstrated the involvement of oxidative metabolism in these resistances. Welling et al. (1974) also showed the importance of oxidative metabolism in OP resistances. The latter reported a strain of houseflies in which the

absence of the malathion specific carboxyesterase enzyme was demonstrated by the failure of TPP to synergise both malathion and malaaxon. In another strain the very high resistance to malathion as well as malaaxon was attributed to both carboxyesterases as well as oxidases. Although sesamex synergised malaaxon, failure to do so with malathion was claimed to be a result of the opposing effects of mfo's in activation and detoxication of this insecticide. Here, although both biochemical studies as well as synergists demonstrated involvement of two resistant factors, genetic evidence had indicated only one (Oppenoorth 1959). The authors therefore assumed the two factors to be closely linked. Harris, et al. (1961) also described a type of malathion resistance involving two genes in houseflies. Georghiou (1969) in his review of the genetics of resistance in houseflies and mosquitoes indicated three major genes to be involved in OP resistance in houseflies, apart from the supplementary effects of one or more other genes. He points out that the one controlling carboxyesterases confers resistance of an incompletely dominant nature.

MATERIALS AND METHODSAnopheline species and populations studied

Species and populations maintained in the laboratory:-

Different populations of a number of anopheline species were maintained in the laboratory. The duration of maintenance of each depended on the nature of the investigations undertaken on it. Populations varied in their ease of maintenance and in this respect fell into three categories.

Readily self-perpetuating without need for artificial mating.

Anopheles stephensi

ST/15 A standard strain derived from a population originating from Delhi, India in about 1947 which was presumed to be "susceptible".

ST/BAR An organochlorine resistant population from Bangalore, South India, colonised in 1971 and kindly provided by the WHO/ICMR Research Unit on Genetic Control of Mosquitoes, Delhi. Sub-colonies BAR/MAL, BAR/43, BAR/33 were derived from it.

ST/ROK An organochlorine resistant population from Roknabad, Minab, Bandar Abbas, South Iran, kindly provided by Dr. A.V. Manouchehri of the Department of Environmental Health, School of Public Health, University of Teheran in 1976. The sub-colonies SM135, SMB6, EM3.16, E136 and STR/DDT were derived from it.

Anopheles culicifacies CUL/PA

An organochlorine resistant population provided by the Pakistan Medical Research Centre, Lahore, Pakistan and derived from the village of Sattoki, South of Lahore from females collected in 1975.



Anopheles albimanus

PALE A susceptible population from Panama maintained in the laboratory in London since 1959, and previously in the United States.

FERNS/RR A multiple resistant population kindly provided by Mr. Su Yung Liu, W.H.O. Entomologist, from El Salvador in 1974.  
Anopheles sacharovi (Soysalli)

A presumably insecticide-susceptible population from Soysalli, Turkey, obtained in 1963.

Species mating in cages to a limited extent but needing supplementary artificial mating to produce adequate numbers.

Anopheles hyrcanus

A multiple resistant population from the Chukurova Plain of Turkey collected in 1975, 1976 and 1977.

Species which would not mate in cages and had to be continuously maintained by the artificial mating technique

Anopheles sacharovi

A multiple resistant population from the Chukurova Plain of Turkey collected in 1974, 1975, 1976 and 1977.

Anopheles culicifacies

CUL/SRL A DDT resistant population from Attanagalla in Kirindiwela, Sri Lanka, kindly provided by the Anti Malaria Campaign, Sri Lanka in 1976.

CUL/IND A malathion resistant population from Wadavali village, Palghar Unit, De Thana, Maharashtra State, India, kindly provided by the National Malaria Eradication Programme of India in 1976.

## Species and populations studied in the field:-

The following populations from the two countries Greece and Turkey have been studied.

Anopheles sacharovi

From the Chukurova Plain of Turkey:- multiresistant populations from the villages of

Asagi Kulak

Kucuk Karatas

Tabaklar

From Greece:-

From the village of Anthili, Lamia Plains

From the villages of Sufli and Poros, Evros.

Anopheles maculipennis

From Osmanjik, Turkey

From villages of Sufli, and Poros in Evros, Greece.

Anopheles superpictus

From the village of Castri, Lamia Plains, Greece.

Anopheles hyrcanus

From the Chukurova Plain of Turkey.

## Rearing:-

The adult mosquitoes were all maintained in an insectary with the temperature ranging between 25-27°C and the relative humidity varying between 70-80%. Larvae were reared in breeding water maintained at a temperature of 28°-30°C. A 12-hour photoperiod in the insectary was maintained with the aid of an automatic time switch.

The population of A. culicifacies, CUL/PA, which had been selected in Pakistan for cage mating was retained in a room where a lighting device was designed to simulate a one hour period each of dusk and dawn, between 6.00 p.m. and 7.00 p.m. and 6.00 a.m. and 7.00 a.m. respectively. At the appropriate time intervals, a gradual decline and an increase in the intensity of light occurred.

The larval rearing was usually accomplished in plastic bowls 12 inches in diameter and 5 inches deep, covered with pieces of bead-weighted netting. In instances where single family rearing was resorted to, enamel bowls measuring 8 inches in diameter, were utilised. The breeding water, tap water, was usually brought to room temperature by filling the bowls a day prior to its use. A piece of turf or a handful of porous soil, preferably with some grass roots, was added to the water as a possible source of nutriment for the first instar larvae.

On an average 200-300 larvae were reared in a bowl. These were fed on ground Farex (a proprietary cereal baby food with added minerals and vitamins), the quantity given at a time depending on the size and the age of the larvae concerned. Sufficient quantities were supplied to ensure a yield of uniform sized larvae and pupae of more or less the same age at the same time, avoiding excess amounts which cause scum formation.

The pupae were drained off into smaller bowls and placed in appropriate cages for either continued maintenance of the populations or for the provision of emerging adults for testing with insecticides. In species where only a limited number of pupae were available, (species requiring artificial mating) they were hand-picked to avoid any accidental loss of material during draining.

Adult populations were retained in cages of sizes varying from 8" x 8" x 8" to 39" x 39" x 39", depending on the density of the respective populations. A continuous supply of approximately

20% glucose solution in the cages was a source of nutriment for the male mosquitoes. The females in addition were fed twice a week on anaesthetized guinea pigs. In species which were either slow or showed reluctance to feed on guinea pigs, feeding on humans was largely resorted to. This was often necessary in the case of A. hyrcanus, and A. sacharovi, while A. culicifacies, CUL/SRL and CUL/IND, were almost always fed on man. The small numbers of adult survivors from insecticide selections were also usually fed on man.

Five inch enamel bowls, lined with filter paper and half-filled with water, were found suitable as oviposition sites for females in cages.

In species which did not mate in the cage, the induced copulation technique, a modification of that described by Baker et al. (1962) was resorted to. Blood fed females and 2 to 3 day old males were usually utilized for mating. A number of males were decapitated each being held by air suction on a micropipette, after which they were glued by the dorsal side of the thorax to the rim of a petri dish. Blood fed females very lightly anaesthetized with ether were each aligned at a time with a male for mating.

A number of attempts were made to establish a self-perpetuating colony of A. culicifacies (CUL/SRL) using cages of three different dimensions (12" x 12" x 12", 18" x 18" x 18", 39" x 39" x 39"). In the last case 500 adults of each sex were released into the cage with no success. In one instance, in the cage measuring 12" x 12" x 12", a single batch of viable eggs were obtained, although a colony could not be established from the progeny of this.

## Chemicals used:

The samples of insecticides and the synergists used were kindly supplied by the following:- Mr. P.R. Chadwick of Wellcome Research Laboratories, Berkhamstead, U.K.; Dr. A.B. Hadaway of Tropical Pesticides Research Unit, Porton Down, Nr. Salisbury, U.K.; Dr. F.J. Oppenoorth of Laboratory for Research on Insecticides, Wageningen, The Netherlands; Dr. R.M. Sawicki of Rothamsted Experimental Station, Harpenden, Hertfordshire, U.K.; Department of Entomology, London School of Hygiene and Tropical Medicine, and the World Health Organization, Geneva. The latter also provided the impregnated papers of DDT, dieldrin, malathion, fenitrothion, fenthion, and propoxur.

InsecticidesOrganophosphatesEthoxy compounds

Aromatic:      chlorphoxim (OMS-1197)

2-chlorophenylglyoxylonitrile oxime O-ester  
with O,O-diethyl phosphorothioate.

phoxim (OMS-1170)

phenylglyoxylonitrile oxime O-ester with  
O,O-diethyl phosphorothioate

parathion

O,O-diethyl-O-p-nitrophenyl phosphorothioate

diazinon

O,O-diethyl 2-isopropyl-6-methyl-4-pyrimidinyl  
phosphorothionate

Aliphatic:      mecabam

S-(N-ethoxy carbonyl-N-methylcarbamoylmethyl)  
diethyl-phosphorothiothionate

Methoxy compoundsAromatic:      iodofenphos (OMS-1211)

O-(2,5-dichloro-4-iodophenyl) O,O-dimethyl  
phosphorothioate

nirimiphos-methyl (OMS-1424)

O,O-dimethyl O-(2-diethylamino-4-methylpyrimidin-  
6-yl) phosphorothiate

fenthion

O,O dimethyl-O-(/4-methylthio/m-tolyl)  
phosphorothioate

fenitrothion

O,O-dimethyl-O-(3-methyl-4-nitrophenyl)  
phosphorothioate

phenthoate

O,O-dimethyl-S-( $\alpha$ -ethoxycarbonylbenzyl)-  
phosphorodithioate

chlorthion

3-chloro-4-nitrophenyl Odimethyl phosphorothionate

Aliphatic:      malathion

O,O-dimethyl-S-/1,2-di(ethoxycarbonyl) ethyl  
phosphorodithioate

bowyl

dimethyl 3-hydroxyglutaconate-dimethyl phosphate

malaaxon

O,O-dimethyl S-/1,2-di(ethoxycarbonyl)ethyl/  
phosphorothioate

dimethoate

dimethyl S-(N-methylcarbamoylmethyl)

phosphorothiolothionate

and

EPN

O-ethyl-O-p-nitrophenyl phenylphosphonothioate

Carbamatespropoxur

2-isopropoxyphenyl N-methyl carbamate

carbaryl

1-naphthyl-N-methylcarbamate

pyrolandimetandimethilan

2-dimethylcarbamoyl-3-methyl-5-pyrazolyl

dimethylcarbamate

3-isopropyl phenyl N-methyl carbamatePyrethroidspermethrin (OMS-1821)

3-phenoxybenzyl di-(1) cis/(3) trans-2,2-

dimethyl-3-(2,2-dichlorovinyl)-cyclopropane-

carboxylate (cis/trans isomers 25:75)

decamethrin (OMS-1998)

(s) alpha-cyano-3-phenoxybenzyl d-cis-3-(2,2-

dibromovinyl)-2,2-dimethyl-cyclopropanecarboxylate

OrganochlorinesDDT

1,1,1-trichloro,-2,2,-bis (p chlorophenyl) ethane

dieldrin

1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,  
5,6,7,8,8a-octa-hydro-1,4-endo-exo-5,8-  
dimethanonaphthalene

Synergists

piperonyl butoxide (PB)

sesamex

octochlorodipropyl ether (S421)

O,O-dimethyl O-phenyl phosphorothioate (SV<sub>1</sub>)

triphenyl phosphate (TFP)

S, S, S-tributyl phosphorotrithioate (DEF)

1,1-bis-(4-chlorophenyl)-2,2,2, trifluoroethanol (F-IMC)

## Preparation of insecticide impregnated papers:

The impregnated papers of DDT, dieldrin, malathion, fenitrothion, fenthion, propoxur were supplied by the World Health Organization. Those of permethrin and decamethrin were made available by Mr. P.R. Chadwick of Wellcome Research Laboratories, Berkhamstead, although initially some of these were prepared also in the laboratory. All other insecticide impregnated papers were prepared according to the following procedure.

Solutions of the desired concentration of each insecticide were prepared in the appropriate solvents. Silicone oil was used as solvent for pyrethroids, the synergists piperonyl butoxide and sesamex, and the carbamate insecticides. With all other compounds dioctyl phthalate was used. The maximum concentration of a particular insecticide required was prepared as the stock solution, from which the necessary dilutions for lower concentrations were obtained.

Whatman's no. 1 filter paper each measuring 12 x 15 cm (180 cm<sup>2</sup>) was used for insecticide impregnation. 0.7 ml of the appropriate



insecticide solution was combined with 1.8 ml of the volatile solvent acetone, and uniformly spread on the filter paper. Before actual use the paper was hung for sufficient time to allow complete evaporation of the acetone.

Establishment of the dosages used:-

The "discriminating dosages" established for the study were based on the minimum concentrations of the respective insecticides which produced a complete mortality on samples of a standard population after exposure for a period of one hour. Although several "susceptible" populations of anophelines were used to some extent, ST/15 the standard population of A. stephensi was used the most.

A series of concentrations of the insecticide concerned was prepared as discussed. Samples of the "susceptible" populations of the species concerned were exposed to these concentrations, each for a period of one hour. Through a process of trial and error the minimum concentration necessary for a complete mortality in the sample was established.

Insecticide susceptibility tests:-

The standard W.H.O. adult mosquito susceptibility test employed, worldwide, particularly under field situations in almost all vector control programmes was adopted in this study. This involved the contact method of confining the mosquitoes to a treated surface, where the insecticide is expected to be gradually picked by the mosquito. The dose picked is presumed to be related to the concentration of the insecticide.

It was hoped that in utilising this technique, any results obtained could be directly related to field situations and therefore be of immediate practical significance.

Most of the testing was performed under laboratory conditions but with some species and populations tests were either carried out solely in the field or in both laboratory and field.

In the laboratory all the investigations made were on adult males and females less than one day old. A relatively constant temperature ( $27^{\circ}\text{C}$  to  $29^{\circ}\text{C}$ ) and a relative humidity (70% to 80%) was maintained during the tests.

In studies made under the field conditions, there was of necessity considerable variation in the physiological stages of the material tested. In addition, since they were carried out at the ambient temperatures, fluctuations in the temperature and relative humidity were encountered. Here the test material consisted entirely of females collected mainly from animal shelters. Although the majority of these were in a blood-fed condition, some semi-gravid and gravids were also used.

The response of different populations to varying insecticides was determined as follows. Samples were exposed to the discriminating concentrations of a particular insecticide at varying periods of exposure ranging from those giving a zero to those giving 100% mortality. Usually these exposure periods fell within 3, 7.5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480 and 960 minutes, the range depending on the susceptibility status of the population and insecticide concerned. For each dose a minimum of 4 replicates, each with an average of 20 to 25 mosquitoes were aimed at, depending on the availability of material. Control tests in duplicate were carried out mainly at the maximum concentration and time of the particular test. As indicated in the description of the standard test procedure mortalities were determined following a 24 hour holding period.

The data were analysed using a computer programme written by Prof. M. Healey (Medical Statistics Department, London School of Hygiene and Tropical Medicine). The programme used the standard maximum likelihood method for fitting a probit curve and provided the  $LT_{50}$  and the value of  $\chi^2$  (Finney 1952). The latter measures the departures of the experimental points from the fitted curve, and significantly large values indicate that the curve is not fitting satisfactorily.

With the exception of the two populations, CUL/IND, of A. culicifacies, and E136 population of A. stephensi used in genetic studies and which were homozygous, all other populations were in varying stages of heterogeneity. Therefore the  $LT_{50}$  values obtained, were used only for approximate comparisons of the resistance levels in different populations.

In populations where a sufficient sample was not available for various reasons, mortalities were determined only at the discriminating dosages established at one hour exposure. Comparison of this value with that in the susceptible population of the same species gives an indication of the proportion of resistant individuals in the population concerned. Similarly increasing trends in the proportion of resistant individuals in a population were estimated using this value of percentage mortality at the discriminating dosage.

#### Laboratory selection for insecticide resistance:-

These selections were made either on laboratory maintained or field collected populations which showed varying levels of the resistances under consideration. The selection procedure adopted, and the extent to which such selections were made in each instance,

depended on the nature of the investigations to be undertaken on the selected population.

The investigations involved the study of the resistance spectra, the establishment of the presence of resistance factors to the selecting agents in the populations concerned, and the ascertaining of the number and mode of inheritance of the genetic factors involved.

Where the selections were made for the study of the resistance spectra, it was continued until higher levels of resistance to the relevant insecticide were obtained. In those made for the establishment of the presence of resistance factors, selections were continued only until the presence or absence of such factors could be confirmed.

For genetic studies, on the other hand, it was necessary to obtain homozygosity for the resistances concerned.

In most instances the procedure involved the mass selection, over a number of generations, of unmated males and females exposed to a concentration and time producing some mortality. The survivors were inbred, artificial mating being necessary in certain instances. In the single family selection, the process of sib selection was adopted. Here, the progeny from individual females were reared independently, tested and survivors inbred. This procedure of sib selection and mating was repeated over a number of successive generations. Detailed accounts of selections made are given in the results section under the particular species concerned.

#### Study of resistance spectra:-

These studies were made on populations already selected for resistance in the field to one or more insecticides, selected to some extent in the field but continued in the laboratory, and in addition the susceptible strains maintained in the laboratory.

Tests on different populations varied with respect to the sample size, number and types of insecticides tested, and according to the availability of test material as well as insecticides. The use of either the serial time for estimating the  $LT_{50}$  values or only the discriminating dosage also depended on the availability of mosquitoes.

The field selected populations were tested either in the field itself on wild caught material or in the laboratory on the progeny of such populations.

Field selected populations tested in the laboratory:-

This included the following populations: A. sacharovi. A. maculipennis and A. hyrcanus from the Chukurova plain, Turkey; A. culicifacies from India, Sri Lanka and Pakistan; and A. stephensi from Iran.

The progenies were derived from eggs received from field material. With the exception of A. stephensi, other species were not easily maintained. In consequence, the material available was limited. In the hope of continuing tests on larger samples later in the field, these tests were therefore of necessity limited to determining the basic information on the nature of resistance patterns, as well as establishing discriminating dosages for certain insecticides in the case of susceptible populations.

Field selected populations studied in the field:-

This included the populations referred to in  
page 48 The A. sacharovi and A. hyrcanus populations from the

Chukurova Plain of Turkey were exposed to all the insecticides listed in section 3.3.1, with the exception of malaoxon and phanthoate. In A. sacharovi mixed populations were sampled consisting of blood-fed, semi-gravid, and gravid females collected resting in animal shelters. The A. hyrcanus tested on the other hand were all blood fed and collected in cattle-baited trap nets. The samples of both species are presumably of varying age groups. Tests were mostly carried out at ambient temperatures which varied from  $31^{\circ}$  -  $33.5^{\circ}\text{C}$  with the relative humidity between 50%-60%. The mortalities at the varying exposure periods of 15, 30, 60, 120, 240 and 360 minutes were determined using the discriminating dosages which were established for the respective insecticides.

Studies on the three species A. sacharovi, A. maculipennis and A. superpictus from the two areas of Greece, were limited to a selected group of insecticides as shown in Tables 34-36, 43-44 and 46. The low mosquito density usually encountered at the period of the year when this study was conducted, was further influenced by the fluctuating weather conditions which prevailed at the time. The limited sample available therefore enabled the use of only the discriminating dosages of one hour exposure in a few instances as in the case of tests on A. superpictus from Castri. The material tested of all three species were mainly blood-fed females, presumably of varying age, collected from animal shelters. However, a few semi-gravids were included in the tests where limited numbers were available. The test temperatures in this instance were comparatively low ranging between  $19^{\circ}$  to  $25^{\circ}\text{C}$  while the relative humidity varied between 52% and 78%.

# Characterisation of detoxication mechanisms by the use of synergists:-

Certain compounds are known to synergise the toxic action of certain insecticides when they are combined with them at a concentration which on their own is non-toxic. So far most of the available information on synergists are cases resulting from inhibition of metabolic detoxication of the insecticides.

In the present study, the characterisation of the possible detoxication mechanisms involved in resistances towards DDT, malathion, fenitrothion and iodofenphos were attempted in a few species using synergists. The latter included piperonyl butoxide (PB), sesamex, octochlorodipropyl ether (S421), all known to inhibit mixed function oxidases, diethyl phenyl phosphorothionate (SV<sub>1</sub>) an inhibitor of both carboxyesterases and oxidases, triphenyl phosphate (TPP) specific for carboxyesterases and S,S,S-tributyl phosphorotrithioate (DEF) that of hydrolytic esterases. In addition, 1,1-bis-(4-chlorophenyl)-2,2,2, trifluoroethano (F-DMC) specific for DDT-dehydrochlorinase (DDT-ase) was used.

With each of these synergists the maximum non-toxic dose was established for the species studied, and the appropriate synergist impregnated papers were prepared.

A sample of the population concerned was first exposed to the non-toxic dose of the relevant synergist, and then exposed to the appropriate insecticide. A comparable test with insecticide only, was also made on a sample of the population. Mortalities were recorded at different periods of exposure for the insecticide alone and for the insecticide-synergist combination. These values were graphically represented on a log-time probit mortality scale. The  $LT_{50}$  values estimated for each was used to calculate the synergistic

ratio (SR). The  $LT_{50}$  value of the insecticide alone divided by that of the synergist-insecticide combination provided this value.

#### Genetic studies:-

The mode of inheritance of malathion resistance and the number of resistance factors involved in A. culicifacies from India was investigated by crossing this strain with the malathion susceptible one from Sri Lanka. The characteristics of the hybrid generation were determined and then backcrosses made to the susceptible strain to determine the number of genetic factors concerned. The same procedure was involved in the study of malathion resistance in A. stephensi.

The  $LT_{50}$ s and  $LT_{90}$ s were calculated from observed data using a programme written for a HP25 calculator. The values were then plotted along with the observed values and the log-probit regression lines shown in figs. 5 and 23 were drawn so as to pass through the calculated  $LT_{50}$  and  $LT_{90}$  values for the relevant populations.



## RESULTS

### A. culicifacies:-

The resistance spectra of the three populations of A. culicifacies. CUL/SRL, CUL/PA and CUL/IND from Sri Lanka, Pakistan and India respectively are represented in Tables 1-4 and fig. 2. All three populations showed high levels of resistance to DDT, the highest being in the strain from Pakistan (CUL/PA). The estimated  $LT_{50}$ 's were 129, 501 and 228 minutes for CUL/SRL, CUL/PA and CUL/IND respectively.

An attempt was made to characterise the detoxication mechanisms involved in the DDT resistance in the CUL/SRL strain using synergists. Pretreatment of the population with piperonyl butoxide (PB) did not enhance the toxicity of this insecticide. This is evidenced from the close proximation of the log time-probit mortality response regression line of DDT and that of DDT following PB treatment (Table 5, fig. 3). The  $LT_{50}$  values were 326 and 312 minutes respectively. This observation suggests that oxidases may not be involved in the DDT resistance in this population. With F-DMC on the other hand increased toxicity of DDT was clearly evident. The  $LT_{50}$  of 326 minutes for DDT was reduced to 61 minutes following treatment with this synergist, showing a synergistic ratio of 5.34. This indicated the involvement of DDT-ase in this resistance.

Towards dieldrin the CUL/SRL population was highly susceptible with the 0.4% concentration producing a 100% mortality at only a 30 minute exposure. The CUL/PA and CUL/IND populations on the other hand were resistant to this insecticide. A complete survival was obtained in both populations with 4.0% dieldrin at one

hour exposure. The CUL/PA in addition was fully resistant to a two hour exposure at this concentration. These two populations could thus be considered almost homozygous for dieldrin resistance. The number tested was however limited.

The CUL/SRL and CUL/PA populations showed susceptibility towards most of the organophosphate (OP) insecticides tested although a few survivors were encountered from most at the discriminating dosages. Thus with 5.0% malathion at one hour exposure, one out of 81, and 5 out of 92 survived in the CUL/SRL and CUL/PA populations respectively. Similarly, two out of 38, and 4 out of 110 adults survived the 1.0% fenitrothion at one hour exposure. In addition in the CUL/PA, all 9 adults exposed to 0.1% diazinon for one hour survived while a sample of 82 in the CUL/SRL gave a complete mortality. This may indicate that the former may be resistant to this insecticide. Similarly a one hour exposure to 0.1% chlorthion which gave a mortality of 92% in the 152 mosquitoes of the CUL/SRL, killed only one adult out of the 4 tested in the CUL/PA strain.

The significance of these survivors in the two populations could not be determined. Failure to receive a lethal dose of the respective insecticides, or reduced effectiveness of the insecticide impregnated papers could be considered to account for these survivors. However, the possibility of these as representing resistant individuals in the populations should not be overlooked.

In contrast to the CUL/SRL and CUL/PA populations, the CUL/IND from Maharashtra State of India exhibited a broad spectrum of high organophosphate resistance. The highest was towards malathion. A complete survival was obtained with an exposure to 5.0% malathion for one hour. Increasing the period of exposure to 8 hours gave only a 24% mortality in 45 adults tested, while with 24 mosquitoes exposed

to 10 hours there was complete survival. The  $LT_{50}$  was estimated at 973 minutes (16.2 hours) compared to the 15 and 21 minutes of the CUL/SRL, and CUL/PA strains respectively. The degree of resistance towards this insecticide was calculated as 65 times that of the CUL/SRL population and 46 times that of the CUL/PA population.

With fenitrothion the CUL/IND strain showed an  $LT_{50}$  of 277 minutes (4.6 hours) while in the CUL/SRL and CUL/PA strains it was 39 and 14 minutes respectively. This indicated a level of resistance 7 and 20 times that of the two more susceptible populations. Levels of resistance in the Indian population 7, 10 and 5 times that of the CUL/SRL population were also encountered towards the insecticides chlorphoxim, pirimiphos-methyl and malaoxon. The percentage mortalities at the discriminating dosages were 37, 4 and 33 compared to 97, 90 and 100 in the susceptible CUL/SRL population. With iodofenphos, chlorthion, and diazinon, where the mortalities were compared at the discriminating dosages only, higher proportions of survivors were recorded in the CUL/IND than in the other two populations.

Towards propoxur and fenthion all three populations can be considered to be susceptible although a few survivors were detected from both CUL/SRL and CUL/IND populations with 2.5% fenthion at one hour exposure.

As in the case of DDT resistance in the CUL/SRL population, synergists were also used in attempts to establish the nature of possible detoxication enzymes involved in malathion resistance in the CUL/IND population. Here in addition to PB, the specific carboxyesterase synergist triphenyl phosphate (TTP) was used.

The involvement of mixed function oxidases (mfo) in the activation of malathion to the toxic malaoxon by conversion of the P - S to P - O bond is well established. Therefore, if this enzyme system is involved only in this activation step, the synergist PB by

either completely inhibiting or reducing the quantity of toxic malaoxon formed may produce antagonism. Even under extreme conditions of incomplete inhibition of activation, no synergism should be anticipated.

As seen in Fig. 4 and Table 6, PB was almost ineffective with malathion. The slight increases or the decreases in the toxicity observed at the different dosages used may not be of significance. However, a similar situation was also observed with a comparable multiresistant population FERN/RR of A. albimanus (Fig. 25) in contrast to the marked antagonistic effect on the malathion selected SM35 population of A. stephensi (Fig. 14) from Iran. In the latter both cross resistance pattern and the use of synergists had pointed to a possible involvement of only a carboxylesterase mechanism. Here, pretreatment with PB showed a continuous antagonism at all the dosages. Therefore lack of antagonism at all the dosages in the two multiple resistant populations of A. culicifacies and A. albimanus, based on a comparable test, may suggest a possible inhibition in these two species, of an additional mechanism that may be involved in the metabolism of malathion since the nature of the response, synergism, antagonism or ineffectiveness may depend on the balance between activation and detoxication and can be influenced by the extent to which either or both of these are inhibited by the synergists. On the other hand the possibility of this varying response being an effect of species difference should not be overlooked. Further, interpretations based on studies using synergists should be made with some reservation in view of certain interactions known to occur between resistance mechanisms themselves as well as between mechanisms and synergists in multiple and multiplicate resistant populations of houseflies.

With TPP, the continuous enhancement of the toxicity of

Table 1. Results of laboratory exposures of *Anopheles culicifacies* - CUL/SHL strain from Attanagalla, Sri Lanka, to various insecticides for varying times

Insecticide	Exposure time in minutes												LT <sub>50</sub> in minutes	χ <sup>2</sup>	DF						
	15			30			45			60						120			240		
	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%	D	T	%
chlorphoxim 4.0%	14	20	70	44	52	85	-	-	-	86	89	97	-	-	-	-	-	-	9	0.30	1
parathion 1.0%	7	7	100	-	-	-	-	-	-	5	5	100	-	-	-	-	-	-	-	-	-
diazinon 0.1%	-	-	-	-	-	-	-	-	-	82	82	100	-	-	-	-	-	-	-	-	-
iodofenphos 10.0%	-	-	-	-	-	-	-	-	-	17	17	100	-	-	-	-	-	-	-	-	-
pirimiphos methyl 1.0%	-	-	-	40	53	76	-	-	-	85	94	90	24	24	100	-	-	-	16	0.61	1
fenthion 2.5%	26	32	81	17	18	94	-	-	-	31	32	95	-	-	-	-	-	-	4	0.20	1
fenitrothion 5.0%	0	10	0	2	12	17	-	-	-	36	38	95	-	-	-	-	-	-	39	-	1
chlorthion 0.1%	4	12	33	6	12	50	-	-	-	140	152	92	-	-	-	-	-	-	23	-	-
malathion 5.0%	12	18	67	22	34	65	23	30	77	80	81	99	-	-	-	-	-	-	15	12.94	2
malaoxon 5.0%	14	47	30	17	30	57	-	-	-	87	87	100	-	-	-	-	-	-	21	7.82	1
dimethoate 1.0%	5	5	100	5	5	100	-	-	-	17	18	94	-	-	-	-	-	-	-	-	-
proponur 0.1%	20	20	100	-	-	-	-	-	-	28	28	100	-	-	-	-	-	-	-	-	-
carbaryl 5.0%	-	-	-	-	-	-	-	-	-	25	28	89	-	-	-	-	-	-	-	-	-
DDT 4.0%	-	-	-	-	-	-	-	-	-	54	445	12	41	95	43	25	29	86	129	0.44	1
dieldrin 0.4%	-	-	-	34	34	100	-	-	-	39	39	100	-	-	-	-	-	-	-	-	-

D = number dead T = total exposed % = percentage mortality

Table 2. Results of laboratory exposures of *Anopheles culicifacies* (CUL/PA) from Lahore,

Pakistan to various insecticides for varying times

Insecticide	Exposure time in minutes												LT <sub>50</sub> in mins	χ <sup>2</sup>	DF												
	15			30			45			60						120			240			360			960		
	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%	D	T	%	D	T	%	D	T	%
chlorphoxim 4.0%	9	26	35	94	107	88	-	-	-	140	140	100	-	-	-	-	-	-	-	-	-	-	-	-	18	0.41	1
parathion 1.0%	-	-	-	-	-	-	-	-	-	0	4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
diazinon 0.1%	-	-	-	-	-	-	-	-	-	0	9	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pirimithos methyl 1.0%	-	-	-	19	20	95	-	-	-	40	42	95	15	15	100	-	-	-	-	-	-	-	-	-	1	0.55	1
fenthion 2.5%	-	-	-	-	-	-	-	-	-	34	34	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
fenitrothion 1.0%	6	13	46	21	23	91	-	-	-	106	110	96	-	-	-	-	-	-	-	-	-	-	-	-	14	1.40	1
chlorthion 0.1%	-	-	-	-	-	-	-	-	-	1	4	25	4	4	100	-	-	-	-	-	-	-	-	-	-	-	-
malathion 5.0%	10	55	18	42	46	91	23	23	100	87	92	95	-	-	-	-	-	-	-	-	-	-	-	-	21	15.60	-
malaoxon 5.0%	1	15	7	16	17	94	-	-	-	16	16	100	-	-	-	-	-	-	-	-	-	-	-	-	21	0.0	1
dimethoate 1.0%	-	-	-	-	-	-	-	-	-	27	28	96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
propoxur 0.1%	12	12	100	-	-	-	-	-	-	44	44	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDT 4.0%	-	-	-	-	-	-	-	-	-	0	72	0	0	28	0	5	46	11	5	20	25	36	41	88	501	0.34	3
dieldrin 4.0%	-	-	-	-	-	-	-	-	-	0	16	0	0	14	0	-	-	-	-	-	-	-	-	-	-	-	-

D = number dead T = total exposed % = percentage mortality

Table 1. Results of laboratory exposures of *Zenaidura macroura* (GML/IRB) from Maharashtra State,

India to various insecticides for varying times

Insecticides	Exposure time in minutes																LT <sub>50</sub> in mins	$\chi^2$	DF												
	15			30			60			90			120			240				360			480			600			1520		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	
chlorphosim 4.0%	1	2	-	3	24	13	24	65	57	-	14	14	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	67	34.45	2	
parathion 1.0%	-	-	-	4	7	57	4	4	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
diazinon 0.1%	-	-	-	-	-	-	0	12	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
iodofenphos 10%	-	-	-	-	-	-	11	22	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60	-	-	-	
pirimetho- methyl 1.0%	1	3	33	-	-	-	5	119	4	-	18	51	35	10	12	85	-	-	-	-	-	-	-	-	-	-	165	961.98	2	-	
fenthion 2.5%	55	42	85	23	24	96	102	114	90	16	16	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(0.3)	3.16	2	-	
fenitrothion 1.0%	-	-	-	-	-	-	14	214	7	-	31	109	28	37	115	32	22	23	96	-	-	-	-	-	-	-	277	22.42	2	-	
chlorthion 0.1%	-	-	-	-	-	-	10	27	57	-	12	12	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
malathion 5.0%	-	-	-	-	-	-	5	215	2	-	2	108	2	10	138	7	19	60	32	11	45	24	0	24	0	16	16	100	973	37.36	5
malaoxon 5.0%	0	50	0	0	98	0	12	36	33	-	8	16	50	-	-	-	-	-	-	-	-	-	-	-	-	-	95	7.78	2	-	
dimethoate 1.0%	4	4	100	-	-	-	17	17	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
propoxur 0.1%	21	54	62	56	28	93	63	63	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	0.24	1	-	
carbaryl 5.0%	-	-	-	-	-	-	10	19	53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
DDT 4.0%	-	-	-	-	-	-	6	90	7	-	1	32	3	6	30	55	18	22	82	-	-	-	-	-	-	-	228	8.41	2	-	
dieldrin 4.0%	-	-	-	-	-	-	0	6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

D = number dead

T = total exposed

% = percentage mortality



Table 1. Results of laboratory exposures of *Jacobsonia culicifurax* (CUL/IRP) from Maharashtra State, India to various insecticides for varying times

Insecticides	Exposure time in minutes												LT <sub>50</sub> in mins	$\chi^2$	DF
	15	30	60	90	120	240	360	480	600	1520					
	D T %	D T %	D T %	D T %	D T %	D T %	D T %	D T %	D T %	D T %					
chlorphosin 4.0%	1 2 -	3 24 13	24 65 37	-	14 14 100	-	-	-	-	-	67	34.43	2		
parathion 1.0%	-	4 7 57	4 4 100	-	-	-	-	-	-	-	-	-	-	-	-
diazinon 0.1%	-	-	0 12 0	-	-	-	-	-	-	-	-	-	-	-	-
iodofenphos 10%	-	-	11 22 50	-	-	-	-	-	-	-	60	-	-	-	-
piridithion- methyl 1.0%	1 3 33	-	5 119 4	-	18 51 35	10 12 83	-	-	-	-	165	961.98	2		
fenthion 2.5%	55 42 83	23 24 96	102 114 90	16 16 100	-	-	-	-	-	-	(0.3)	3.16	2		
fenitrothion 1.0%	-	-	14 214 7	-	51 109 28	37 115 32	22 23 96	-	-	-	277	22.42	2		
chlorthion 0.1%	-	-	10 27 37	-	12 12 100	-	-	-	-	-	-	-	-	-	-
malathion 5.0%	-	-	5 215 2	-	2 108 2	10 158 7	19 60 32	11 45 24	0 24 0	16 16 100	973	37.36	5		
malaoxon 5.0%	0 30 0	0 98 0	12 96 33	-	8 16 50	-	-	-	-	-	95	7.78	2		
dimethoate 1.0%	4 4 100	-	17 17 100	-	-	-	-	-	-	-	-	-	-	-	-
propanur 0.1%	21 34 62	36 28 93	63 63 100	-	-	-	-	-	-	-	13	0.24	1		
carbaryl 5.0%	-	-	10 19 53	-	-	-	-	-	-	-	-	-	-	-	-
DDT 4.0%	-	-	6 90 7	-	1 32 3	16 30 53	18 22 82	-	-	-	228	8.41	2		
dieldrin 4.0%	-	-	0 6 0	-	-	-	-	-	-	-	-	-	-	-	-

D = number dead T = total exposed % = percentage mortality

Table 3. Results of laboratory exposures of *Anopheles culicifacies* (CUL/IND) from Maharashtra State, India to various insecticides for varying times

Insecticides	Exposure time in minutes												LT <sub>50</sub> in mins	$\chi^2$	DF
	15	30	60	90	120	240	360	480	600	1320					
	D T %	D T %	D T %	D T %	D T %	D T %	D T %	D T %	D T %	D T %	D T %				
chlorpyrifos 4.0%	1 2 -	3 24 13	24 65 37	-	14 14 100	-	-	-	-	-	-	67	34.43	2	
parathion 1.0%	-	4 7 57	4 4 100	-	-	-	-	-	-	-	-	-	-	-	
diazinon 0.1%	-	-	0 12 0	-	-	-	-	-	-	-	-	-	-	-	
iodofenphos 10%	-	-	11 22 50	-	-	-	-	-	-	-	-	60	-	-	
pirimithos- methyl 1.0%	1 3 33	-	5 119 4	-	18 51 35	10 12 85	-	-	-	-	-	165	961.98	2	
fenthion 2.5%	35 42 83	23 24 96	102 114 90	16 16 100	-	-	-	-	-	-	-	(0.5)	5.16	2	
fenitrothion 1.0%	-	-	14 214 7	-	31 109 28	37 115 32	22 23 96	-	-	-	-	277	22.42	2	
chlorfenthion 0.1%	-	-	10 27 57	-	12 12 100	-	-	-	-	-	-	-	-	-	
malathion 5.0%	-	-	5 215 2	-	2 108 2	10 138 7	19 60 32	1 45 24	0 24 0	6 16 100	-	973	37.36	5	
malathion 5.0%	0 50 0	0 98 0	12 36 33	-	8 16 50	-	-	-	-	-	-	95	7.78	2	
dimethoate 1.0%	4 4 100	-	17 17 100	-	-	-	-	-	-	-	-	-	-	-	
propoxur 0.1%	21 34 62	6 28 93	63 63 100	-	-	-	-	-	-	-	-	13	0.24	1	
carbaryl 5.0%	-	-	10 19 53	-	-	-	-	-	-	-	-	-	-	-	
DDT 4.0%	-	-	6 90 7	-	1 32 3	16 30 53	18 22 82	-	-	-	-	228	8.41	2	
dieldrin 4.0%	-	-	0 6 0	-	-	-	-	-	-	-	-	-	-	-	

D = number dead T = total exposed % = percentage mortality

Table 4. The percentage mortalities at discriminating dosages, and the  $Lt_{50}$  values of populations of *A. culicifacies* CUL/SRL, CUL/PA and CUL/IND from Sri Lanka, Pakistan and India respectively exposed to different insecticides. Rf = resistant factor in terms of CUL/SRL strain

Insecticide	CUL/SRL		CUL/PA		Rf	CUL/IND		Rf
	% mortality	$Lt_{50}$	% mortality	$Lt_{50}$		% mortality	$Lt_{50}$	
chlorphoxim 4.0%	97	9	100	18		37	67	7(4)*
parathion 1.0%	100	-	0	-		100	-	
diazinon 0.1%	100	-	0	-		0	-	
iodofenphos 10%	100	-	-	-		50	60	
pirimiphor-methyl 1.0%	90	16	95	1		4	165	10(165)*
fenthion 2.5%	95	4	100	-		90	0.3	
fenitrothion 1.0%	95	39	96	14		7	277	7(20)*
chlorthion 0.1%	92	23	25	-		37	-	
malathion 5.0%	99	15	95	21		2	973	65(46)*
malaoxon 5.0%	100	21	100	21		33	95	5(5)*
dimethoate 1.0%	94	-	96	-		100	-	
propoxur 0.1%	100	-	100	-		100	13	
carbaryl 5.0%	89	-	-	-		53	-	
DDT 4.0%	12	129	0	501	4	7	228	2
dieldrin 4.0%	100**	-	0	-		0	-	

( )\* = Rf compared to CUL/PA strain

\*\* = 0.4%

Table 4. The percentage mortalities at discriminating dosages, and the  $Lt_{50}$  values of populations of *A. culicifacies* CUL/SRL, CUL/PA and CUL/IND from Sri Lanka, Pakistan and India respectively exposed to different insecticides. Rf - resistant factor in terms of CUL/SRL strain

Insecticide	CUL/SRL		CUL/PA			CUL/IND		
	% mortality	$Lt_{50}$	% mortality	$Lt_{50}$	Rf	% mortality	$Lt_{50}$	Rf
chlorphoxin 4.0%	97	9	100	18		37	67	7(4)*
parathion 1.0%	100	-	0	-		100	-	
diazinon 0.1%	100	-	0	-		0	-	
iodofenphos 10%	100	-	-	-		50	60	
pirimiphos-methyl 1.0%	90	16	95	1		4	165	10(165)*
fenthion 2.5%	95	4	100	-		90	0.3	
fenitrothion 1.0%	95	39	96	14		7	277	7(20)*
chlorthion 0.1%	92	23	25	-		37	-	
malathion 5.0%	99	15	95	21		2	973	65(46)*
malaoxon 5.0%	100	21	100	21		33	95	5(5)*
dimethoate 1.0%	94	-	96	-		100	-	
propoxur 0.1%	100	-	100	-		100	13	
carbaryl 5.0%	89	-	-	-		53	-	
DDT 4.0%	12	129	0	501	4	7	228	2
dieldrin 4.0%	100**	-	0	-		0	-	

(\*) - Rf compared to CUL/PA strain

\*\* = 0.4%

Fig.2

Resistance spectra of two populations of *A. culicifacies* from Attanagalla, Sri Lanka (CUL/SRL) and Maharashtra State, India (CUL/IND)

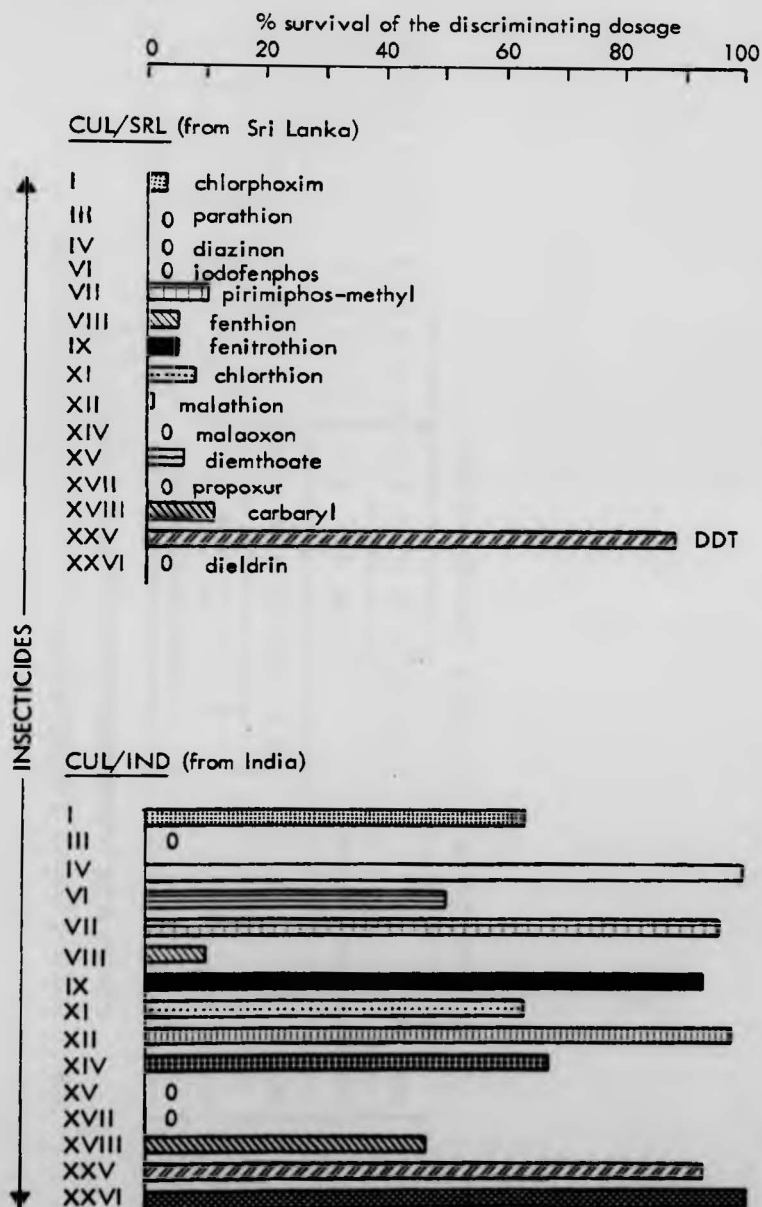


Table 5. Results of exposures of CUL/SRL population of A. culicifacies from Sri Lanka to DDT

alone and to DDT following pre-treatment with piperonyl butoxide and P-DMC

Compounds and concentration	Exposure time in minutes															LT <sub>50</sub>	χ <sup>2</sup>	SR
	60			120			240			300			360					
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%			
DDT 2.0%	0	24	0	6	36	17	9	63	14	14	48	29	40	48	83	326	36.4	-
Piperonyl-butoxide + DDT 2.0%	-			1	23	4	5	27	19	13	37	35	20	24	83	312	12.7	1.04
FIMC + DDT 2.0%	11	22	50	13	19	68	11	13	85	16	20	80	17	18	94	61	1.30	5.34

D = number tested, T = total exposed, % = percentage mortality

Fig.3

The mortality relationship in *A. culicifacies* (CUL/SRL) exposed to DDT alone and DDT after previous exposure to the synergists F-DMC, and PB.

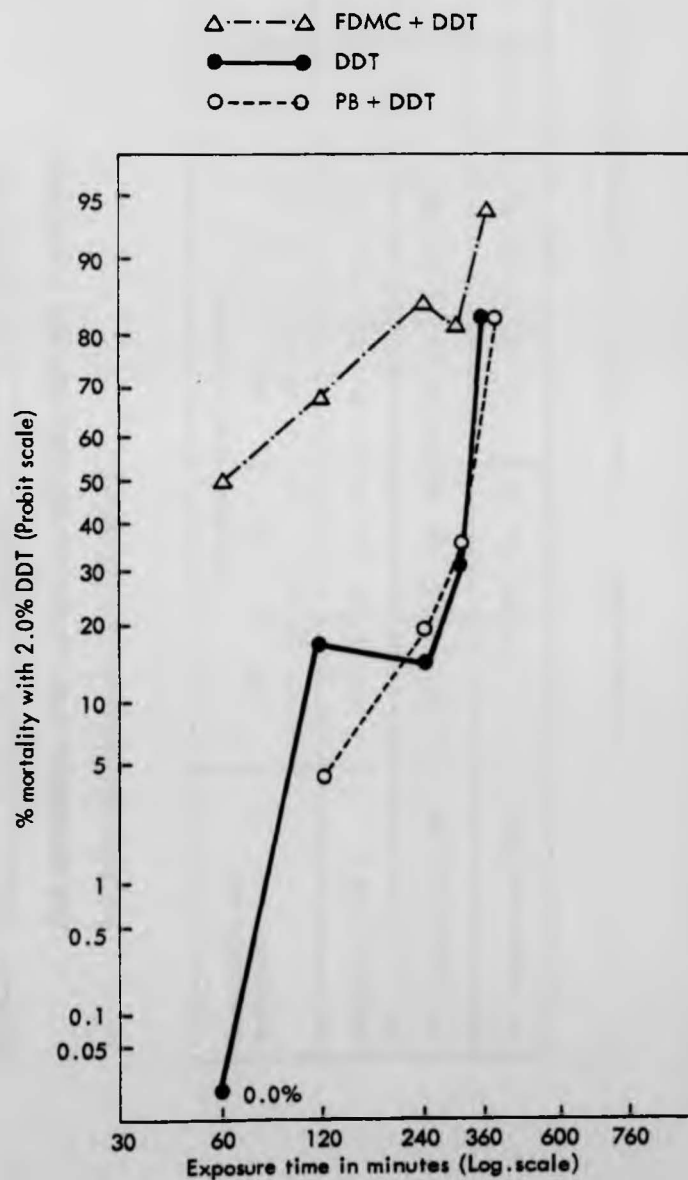


Table 6. Results of exposures of the CUL/IND population of *A. culicifacies* from Maharashtra State, India, to malathion alone and to malathion following pretreatments with TPP and PB

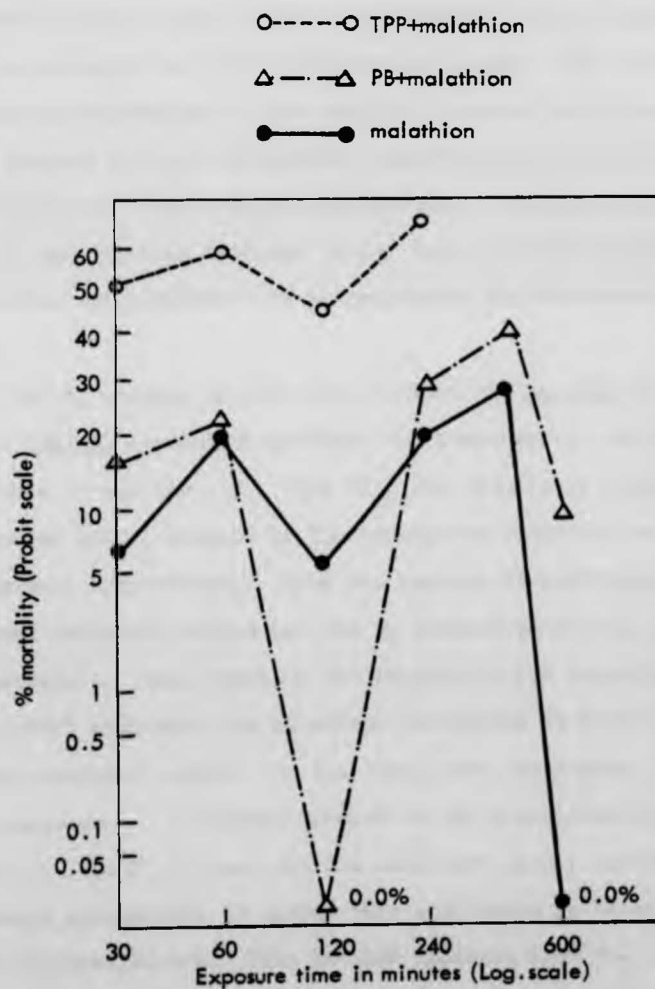
Insecticide and synergist	Exposure time in minutes																	
	30			60			120			240			420			600		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%
malathion 5.0%	2	36	6	30	161	19	2	39	5	9	47	19	7	26	27	0	16	0
TPP + malathion 5.0%	34	67	51	51	88	58	29	66	44	60	93	65	-			-		
PB + malathion 5.0%	2	13	15	26	124	21	0	15	0	36	123	29	14	36	39	2	22	9

D = number dead    T = total exposed    % = percentage mortality



Fig.4

The mortality relationship in *A.culicifacies* (CUL/IND) exposed to malathion alone and to malathion after previous exposure to the synergists TPP and PB.



malathion seen at all the dosages tested, is suggestive of the involvement of carboxylesterases in this resistance.

Studies were also made to determine the mode of inheritance as well as the number of genetic factors involved in the malathion resistance in this CUL/IND population.

The progeny from the eggs received from the Maharashtra State, already showed a high level of resistance to this insecticide. There was no mortality at the discriminating dosage. The population was continuously maintained by the artificial mating technique. The entire progeny in each successive generation were subjected to malathion pressure at the discriminating dosage. Since there was virtually no susceptibles detected at any time over the generations, this population was considered to be homozygous for malathion resistance.

The  $F_1$  progeny of the cross between the CUL/IND and the susceptible CUL/SRL population produced the response to 5.0% malathion shown in Table 7 and fig. 5. The  $LT_{50}$  was 39 minutes compared to the 705 minutes and 22 minutes of the homozygous resistant and susceptible parents respectively. Thus the degrees of resistance shown by the parent resistant strain and the  $F_1$  generation were 32 and 17 times respectively. This based on the interpretation suggested by Georgiou (1969) indicates the malathion resistance to be of an incompletely dominant nature. On the other hand considering the identical response (2.0%) encountered at the discriminating dosage of malathion in the  $F_1$  progeny and the resistant parent respectively, it may be more appropriate to assume this resistance as being of a completely dominant nature. This is also apparent from the nature of the regression line of the two genotypes particularly if compared with the situation in the malathion resistant E136 of A. stephensi (fig. 23).

Table 7 . Results of exposures of the resistant CUL/IND strain of  
A. culicifacies from India, the susceptible CUL/SRL  
strain from Sri Lanka and hybrids between them to  
5.0% malathion for varying periods of time

Exposure time in minutes	CUL/IND			CUL/SRL			CUL/IND x CUL/SRL		
	D	T	%	D	T	%	D	T	%
15	-	-	-	12	18	67	-	-	-
30	-	-	-	22	34	65	-	-	-
45	-	-	-	23	30	77	-	-	-
60	5	215	2	80	81	99	3	155	2
120	2	108	2	-	-	-	4	75	5
180	-	-	-	-	-	-	3	33	9
240	10	138	7	-	-	-	61	131	47
360	19	60	32	-	-	-	2	33	6
480	11	45	24	-	-	-	-	-	-
600	0	24	0	-	-	-	-	-	-
1320	16	16	100	-	-	-	-	-	-
LT <sub>50</sub> (mins)	705			22			369		
LT <sub>90</sub> (mins)	2867			48			1026		
Slope	2.10			3.65			2.88		

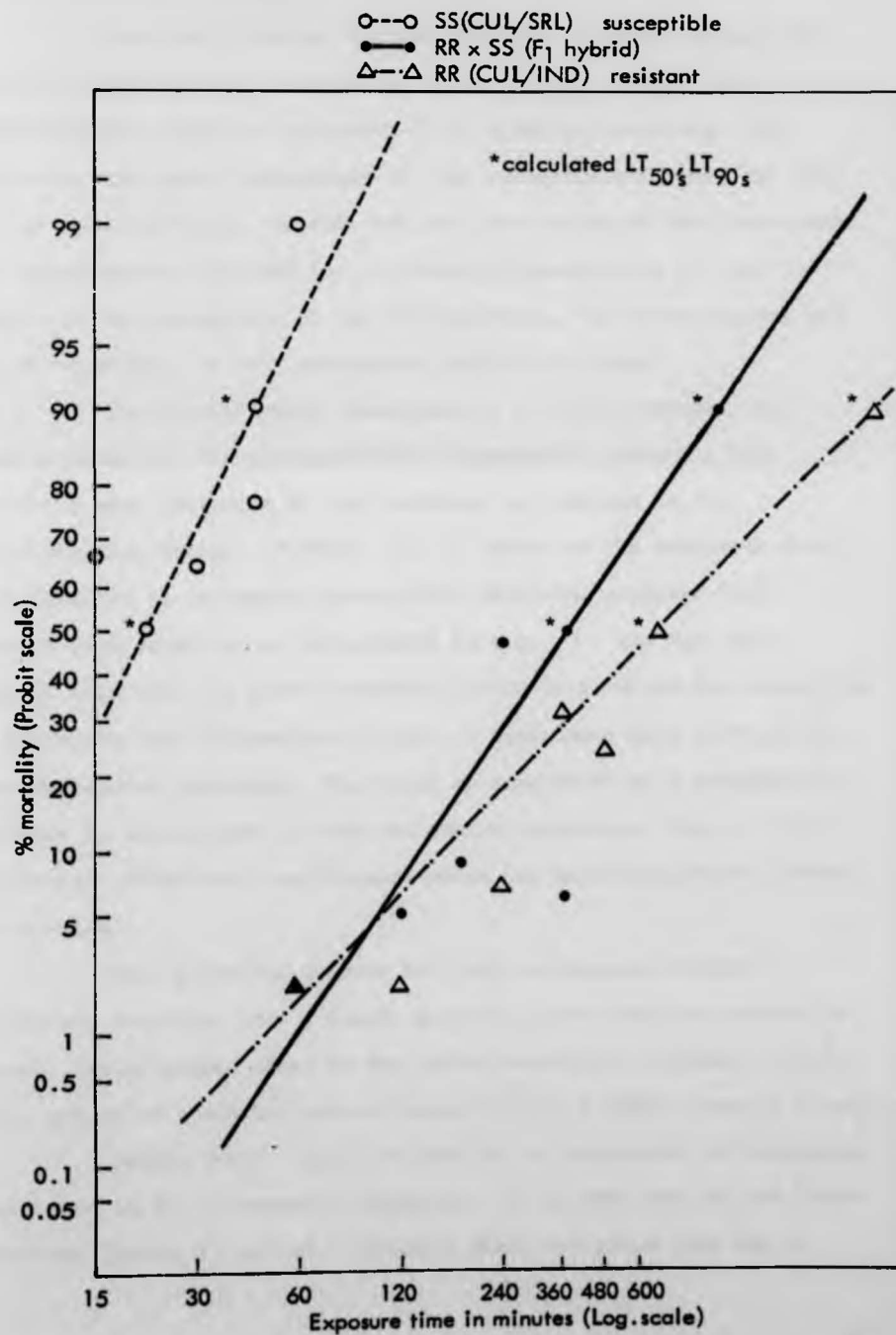
D - number dead

T - total tested

% - percentage mortality

Fig.5

Log-time probit mortality regression lines for *A.culicifacies* from India (CUL/IND), Sri Lanka (CUL/SRL) and hybrids between them exposed to malathion.



In the establishment of the number of major genetic factors contributing to this resistance a procedure originally proposed by Wright (1952) was followed.

Here the offspring derived from the backcross between the  $F_1$  heterozygote and the susceptible parent were exposed to the discriminating dosage of malathion (i.e. 5.0% for one hour). The survivors were again backcrossed to the susceptible parent, the offspring being similarly treated with the same dosage of the insecticide. The procedure was repeated for 3 successive generations so that the nature of the segregation of the two genotypes, the heterozygotes and the susceptibles, in each generation could be followed.

For monofactorial inheritance a 1:1 ratio between the heterozygotes and the susceptibles is expected to produce a 50% mortality when offspring of the backcross are exposed to the discriminating dosage. Further this 1:1 ratio in the genotypes should be maintained in successive generations following repeated backcrosses with selection as illustrated in fig. 6. On the other hand if more than one major resistant factor is involved the proportion of genotypes with intermediate levels of resistance will increase in each successive backcross. This will be manifested by a progressive increase in mortalities in each successive backcross. Figs. 7 and 8 illustrate situations anticipated where two major resistance factors are involved.

This procedure however will not distinguish between resistance resulting from a single genetic factor from that caused by closely linked genes. Thus in the latter event such studies on backcross progenies would indicate evidence of only a single genetic factor.

Tables 8-10 show the results of segregation of malathion resistance in the 3 backcross progenies. It is seen that in the first backcross (Table 8) out of 5 families where the yield from egg to

Fig.6

Schematic representation of repeated backcrossing with selection to prove monofactorial inheritance (Wright 1952)

M = resistant allele  
m = susceptible allele

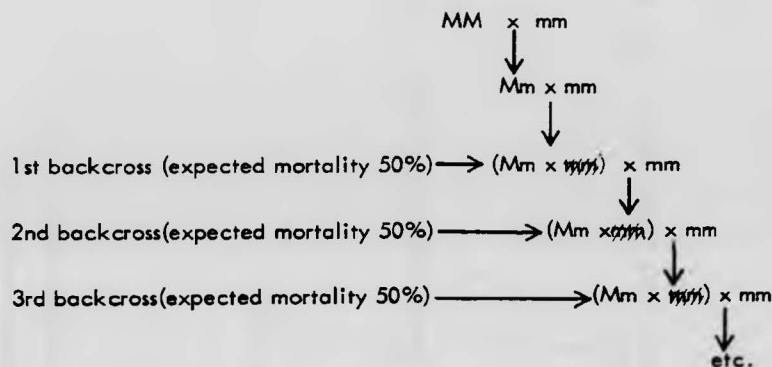


Fig.7

Schematic representation of bi-factorial inheritance assuming that both resistance factors are required for survival at the discriminating dosage.

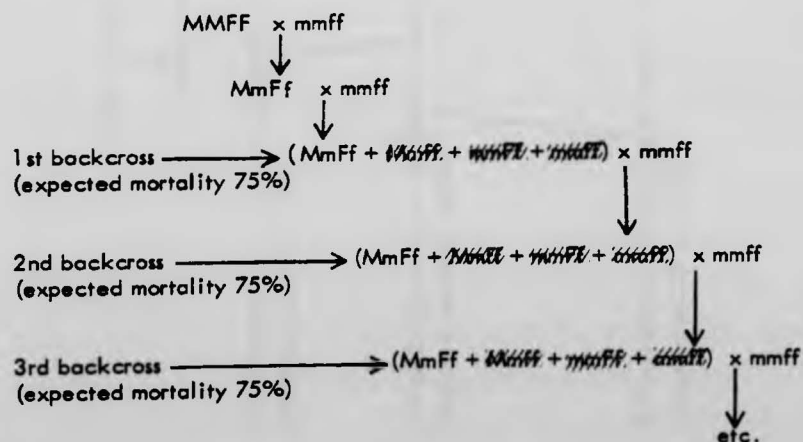
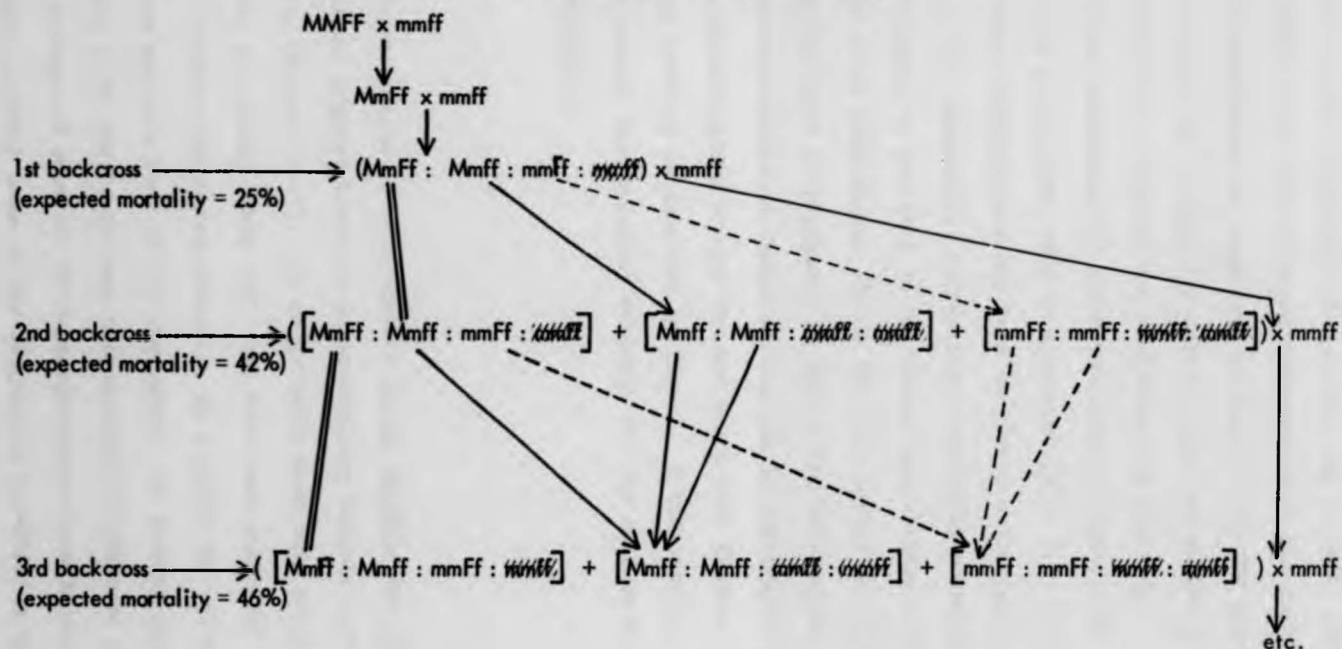


Fig.8

Schematic representation of bifactorial inheritance assuming each resistant factor on its own is sufficient for survival on the discriminating dosage.

M, F = resistant alleles (same as  $R_1$   $R_2$ )

mf = susceptible alleles (same as  $S_1$   $S_2$ )



adult was less than 75%, two show a significant departure from a 1:1 ratio. On the other hand 6 out of 7 families with higher egg to adult yields (75%-90%) were in agreement with this expected segregation of the genotypes for monofactorial inheritance as suggested in fig. 6. In the second backcross (Table 9), out of 15 families tested, only one showed a departure from this ratio. Results are available for only two families in the third backcross, of which one shows a significant departure, while the pooled data were in agreement ( $\chi^2 = 2.5$ ,  $p > 0.1$ ).

Although these observations may tend to favour a monofactorial mode of inheritance in this resistance, increasing mortalities observed in successive backcrosses, in addition to the actual mortalities recorded in the second and third backcrosses (Table 10) also appear to favour the expected segregation ratio as suggested in fig. 8 for bifactorial inheritance where each resistant factor on its own is sufficient for survival on the discriminating dosage but not where both factors are required for this survival as depicted in fig. 7. A bifactorial interpretation would support the synergist evidence for the existence of two detoxication mechanisms.

#### A. stephensi

Of the three strains of this species ST/15, ST/BAR, and ST/ROK, the ST/15 showed the highest level of susceptibility towards most of the insecticides. (Tables 11-13). It is probable that this population, maintained in the laboratory since 1947, may have been collected prior to exposure to extensive selection pressure, as a result of which resistant factors may have been in low frequency. On testing large samples, however, a few survivors were occasionally encountered at the discriminating dosages of some of the organophosphate insecticides. Failure to receive a lethal dose of the respective insecticide by resting on the wire netting of the exposure tubes, reduced effectiveness of the impregnated paper, or even the possible effects of slight fluctuating test temperatures to which this species was found to be sensitive are all possibilities that may account for these survivors. However the possible existence of



Table 8. Single family results of exposures to the discriminating dosage of malathion of the offspring of the first backcross of the hybrid (resistant Indian x susceptible Sri Lanka) to the susceptible Sri Lanka population (*A. culicifacies*)

Batch no.	% yield no. pupae/no. eggs	Total no. tested	No. susceptible	% mortality	$\chi^2$ (1:1 expectation)	P
	<u><math>\leq 75\%</math> yield</u>					
1	68	22	9	41	0.36	$> 0.10$
2	17	45	11	24	5.90	$< 0.01$
3	40	55	24	44	0.45	$> 0.10$
4	59	20	6	30	1.60	$> 0.10$
5	53	142	49	35	6.80	$< 0.01$
	<u><math>&gt; 75\%</math> yield</u>					
6	81	43	17	40	0.94	$> 0.10$
7	89	33	15	46	0.14	$> 0.10$
8	78	35	21	60	0.70	$> 0.10$
9	80	36	12	33	2.00	$> 0.10$
10	80	84	28	33	4.70	$\leq 0.05$
11	90	71	32	45	0.35	$> 0.10$
12	75	60	25	42	1.20	$> 0.10$

Table 9. Single family results of exposures to the discriminating dosage of malathion of the second backcross progeny involving *A. culicifacies* populations from India and Sri Lanka

Batch no.	% yield no. pupae/no. eggs	Total no. tested	No. susceptible	% mortality	$\chi^2$ (1:1 expected)	P
<u>&lt; 70% yield</u>						
1	42	38	23	61	0.84	> 0.10
2	48	79	35	44	0.51	> 0.10
3	67	122	55	45	0.59	> 0.10
<u>&gt; 70% yield</u>						
4	74	28	13	46	0.07	> 0.10
5	70	39	14	36	1.60	> 0.10
6	74	176	53	30	13.90	< 0.01
7	72	64	35	55	0.28	> 0.10
8	73	86	47	53	0.21	> 0.10
9	72	132	57	43	1.20	> 0.10
10	74	35	20	57	0.36	> 0.10
11	75	42	16	38	1.20	> 0.10
12	89	86	43	50	0.00	-
13	96	24	5	21	4.10	< 0.05
14	-	37	12	32	2.28	> 0.10
15	98	63	25	40	1.34	> 0.10

Table 10. Summary of the results of exposures of the progenies of three consecutive backcrosses with selection (involving the Indian resistant and Sri Lankan susceptible populations of *A. culicifacies*) to the discriminating dosage of malathion.

	first backcross	second backcross	third backcross
% mortality in batches > 75% yield $\chi^2$	42 (293) 3.80	42 (814) 11.00	46 (55) 0.23
% mortality in batches < 75% yield $\chi^2$	36 (353) 14.45	44 (239) 0.35	83 (41) 8.90
% mortality all batches combined $\chi^2$	39 (646) 16.95	43 (1053) 10.26	62 (96) 2.52

Table 10. Summary of the results of exposures of the progenies of three consecutive backcrosses with selection (involving the Indian resistant and Sri Lankan susceptible populations of *A. culicifacies*) to the discriminating dosage of malathion.

	first backcross	second backcross	third backcross
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% mortality all batches combined $\chi^2$	39 (646) 16.95	43 (1053) 10.26	62 (96) 2.52

resistant individuals should not be ruled out as no attempt was made to confirm this fact.

With 4.0% DDT, complete mortality was not obtained at one hour exposure, while only 87% mortality was recorded at an exposure for 2 hours. In addition this population showed a tolerance towards the two pyrethroid insecticides, permethrin and decamethrin (fig.s. 12 & 13A) when compared with a population SM35, of the same species originating from ST/ROK from Iran. The  $LT_{50}$  of ST/15 were 44 and 61 minutes with permethrin and decamethrin respectively compared with 29 and 26 minutes in the other population. It is possible that these tolerances to both DDT and pyrethroids may be attributable to a common factor.

Both ST/BAR and ST/ROK showed high levels of resistance to DDT, this being extremely high in the case of ST/BAR. Here a complete survival was obtained at an exposure to 4.0% DDT for 2 hours. The  $LT_{50}$  was 658 minutes compared with 35 minutes for ST/15. This is estimated as a 19-fold increase in the resistance level. In ST/ROK the  $LT_{50}$  of 152 minutes shows a resistance level 4 times that of ST/15.

The only organophosphate towards which a considerable level of resistance was shown by ST/BAR was iodofenphos (Table 12 fig. 13B). Here the mortality at one hour exposure to a 10% concentration was 48% compared with 98% in the ST/15 strain. The  $LT_{50}$  of 63 minutes in ST/BAR indicated a 7-fold increase in the resistance level when compared with the 9 minutes in the ST/15.

A few survivors were also encountered at the discriminating dosages of most of the organophosphate insecticides in the ST/BAR population. This was particularly so with malathion, malaoxon, phenthoate, fenitrothion and propoxur. Table 12 and fig. 13B show the  $LT_{50}$  values to be considerably higher than those of the ST/15 population. An attempt was made to determine the significance of these survivors. The survivors of malathion, fenitrothion and propoxur were independently selected with the discriminating dosages of the respective insecticides.

As the numbers surviving the exposures were often low, selections in each successive generation was not always possible. As shown in Table 14 and fig. 9 continued selection of malathion survivors had increased the resistance level from 99% mortality in the parent population to 43% in the selected sample. The continued selection of fenitrothion survivors similarly showed a reduction in the mortality from 98% in the parent population to 25% following selection. With propoxur the percentage mortality of 96% in the original population was reduced to 63% after selection. Here however the selecting dosage was a 30 minute exposure to 0.1% concentration unlike the one hour in each of the others. These changes in the resistance levels are indications of the presence of individuals resistant to these insecticides in the ST/BAR population. Whether this is applicable to the survivors of exposures to other insecticides is not known and was not investigated.

In the ST/ROK population from Iran the eggs received from the field were few in number and thus assumed to represent a restricted gene pool. Therefore attempts were made to prevent any further limitations on this aspect through loss of any material during the maintenance of the populations or the selection for insecticide resistance.

The progeny from the eggs were maintained over a number of generations to increase the population size, ensuring that no material was discarded at any stage in the development and maintenance. When the desired population size was available the base line susceptibility levels were determined on a sample of the population both in terms of  $LT_{50}$  as well as mortalities at the discriminating dosages, for the available insecticides. With pirimiphos-methyl however only a one hour exposure was made. A comparison was made on the susceptible ST/15.

on a similar basis. As shown in Table 13 the ST/ROK population was resistant to DDT, giving a 9% mortality at one hour exposure to 4.0% DDT. With 5.0% malathion at one hour exposure 3 survivors were encountered out of 246 adults tested showing a mortality of 99%. The estimated  $LT_{50}$  was 9 minutes, which was in fact lower than the 15 minutes shown by the ST/15 population. A complete susceptibility (100% mortality) was obtained on testing 112 adults on 1.0% fenitrothion for one hour. At this dosage, 97% and 100% mortalities at exposure periods of 45 and 30 minutes respectively occurred with samples of 34 and 36 mosquitoes of ST/15. The  $LT_{50}$  was calculated as 16 minutes, approximating the 15 minutes of the ST/15. However, the same population of ST/ROK was also tested at the same time on a set of fenitrothion impregnated papers which had been previously used but gave a 100% kill of 174 adults of the ST/15 strain. This produced only a 77% mortality out of 491 ST/ROK mosquitoes tested. The  $LT_{50}$  of ST/ROK in this instance was 37 minutes, twice that of ST/15. In addition, 1.0% pirimiphos-methyl at an exposure of one hour gave a 79% kill compared with the 95% of the susceptible population.

Towards fenthion and propoxur the population was highly susceptible. A 30 minute exposure to 0.1% propoxur and a 15 minute exposure to 2.5% fenthion was sufficient to produce a complete mortality.

Having established the baseline susceptibility levels, the samples from the ST/ROK population were then used for independent selection for resistance to malathion, fenitrothion, propoxur and DDT.

#### Selection for malathion resistance:

The selection procedure usually adopted on the basis of constant mortality and constant dosage was not adhered to. Instead, it was conducted on a gradual basis.

Table II. Results of laboratory exposures of *Anopheles stephensi* - 87/15 strain from Delhi  
India to various insecticides for varying times

Insecticides	Exposure time in minutes																		LT <sub>50</sub> in min	χ <sup>2</sup>	DF						
	3			7.5			15			30			45			60						90			120		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%	D	T	%
chlorphoxim 4.0%	-	-	-	-	-	-	41	126	57	16	22	73	31	133	99	403	424	95	-	-	-	-	-	-	18	11.28	2
phoxim 2.5%	-	16	67	24	114	259	44	109	190	57	172	471	79	314	321	98	-	-	-	-	-	-	-	-	19	45.83	3
iodofenphos 10.0%	-	42	86	49	52	72	72	174	227	77	77	281	99	366	374	98	-	-	-	-	-	-	-	-	9	40.45	3
pirimiphos methyl 1.0%	-	-	-	-	-	-	73	107	68	-	-	-	101	145	70	291	316	92	-	122	140	87	-	-	5	29.74	2
fenthion 2.5%	-	15	86	17	80	85	94	95	95	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	0.00	1
fenitrothion 1.0%	-	16	209	8	303	536	72	550	579	95	30	231	100	321	322	100	-	-	-	-	-	-	-	-	12	26.07	3
phenothate 10.0%	-	11	144	8	158	218	73	177	211	84	107	445	92	732	769	95	-	-	-	-	-	-	-	-	14	51.23	3
malathion 5%	-	71	297	24	318	671	47	726	932	78	809	622	95	1036	1054	98	-	-	-	-	-	-	-	-	15	32.66	3
malaxon 5.0%	-	6	53	11	65	163	40	-	-	-	85	290	98	222	222	100	-	-	-	73	73	100	-	-	16	4.88	3
propoxur 0.1%	20	105	2	39	95	41	90	94	96	70	70	100	-	-	-	-	-	-	-	-	-	-	-	-	7	20.62	2
permethrin 0.2%	-	-	-	-	-	-	8	49	16	59	212	27	70	139	50	248	368	67	-	82	93	88	-	-	44	5.60	3
decamethrin 0.001%	-	-	-	-	-	-	-	1	89	1	30	84	36	61	96	64	43	71	61	-	-	-	-	-	61	27.2	2
DDE 4.0%	17	49	35	9	30	24	53	165	32	49	155	32	96	126	76	78	179	44	-	104	119	87	-	-	35	91.63	5

D = number dead      T = total exposed      % percentage mortality



Table 12. Results of laboratory exposures of *Anopheles stephensi* (SP/BAU) from Bangalore.

India to various insecticides for varying times

Insecticides	Exposure time in minutes												LT <sub>50</sub> in mins	$\chi^2$	DF
	7.5 D T %	15 D T %	30 D T %	45 D T %	60 D T %	90 D T %	120 D T %	240 D T %	360 D T %	480 D T %	960 D T %				
chlorphoxim 4.0%	10 95 11	22 49 45	22 95 23	174 225 77	187 200 94	-	-	-	-	-	-	20	84.84	3	
phoxim 2.5%	-	2 78 9	74 110 67	62 63 98	21 21 100	-	-	-	-	-	-	26	0.42	2	
iodofenphos 10.0%	-	-	2 25 8	9 78 12	57 119 48	-	-	-	-	-	-	63	6.92	1	
pirimiphos methyl 1.0%	61 62 98	126 133 95	117 167 70	68 68 10	73 73 100	-	-	-	-	-	-	-	-	-	
fenthion 2.5%	15 86 17	80 85 94	95 95 100	-	-	-	-	-	-	-	-	10	0.0	1	
fenitrothion 1.0%	-	18 138 13	98 248 40	237 274 87	164 167 98	-	-	-	-	-	-	30	32.31	2	
phenthoate 10.0%	-	-	34 78 44	61 135 45	156 216 72	-	-	-	-	-	-	40	8.91	1	
malathion 5.0%	-	14 135 10	45 133 34	52 107 49	297 301 99	-	-	-	-	-	-	33	80.95	2	
malaoxon 0.1%	-	10 129 8	28 184 15	50 96 52	96 120 80	-	73 73 100	-	-	-	-	43	31.80	3	
propoxur 0.1%	54 191 28	170 179 95	245 252 97	-	23 24 96	-	-	-	-	-	-	9	134.52	2	
DDP 4.0%	-	-	-	-	1 126 1	-	0 135 0	9 179 5	5 64 9	2 69 3	65 70 93	658	4	3	

D = number dead T = total exposed % = percentage mortality

**Table 13.** Results of laboratory exposures of the unselected population of  
*Anopheles stephensi* (ST/ROK) from Iran, to various insecticides for varying times

Insecticides	Exposure time in minutes															LT <sub>50</sub> in mins	χ <sup>2</sup>	DF												
	15			30			45			60			120						180			240			360			480		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%	D	T	%	D	T	%	D	T	%
fenthion	29	29	100	29	29	100	-	-	-	14	14	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
fenitrothion*	1	33	3	30	71	43	-	-	-	377	491	77	-	-	-	-	-	-	-	-	-	-	-	-	37	2.00	1	-	-	
fenitrothion	12	29	41	36	36	100	33	34	97	112	112	100	-	-	-	-	-	-	-	-	-	-	-	-	16	8.32	2	-	-	
malathion	188	257	73	152	227	67	-	-	-	243	246	99	-	-	-	-	-	-	-	-	-	-	-	-	9	56.56	1	-	-	
propoxur	65	89	73	89	89	100	86	86	100	105	105	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
DDT	3	42	7	6	57	11	-	-	-	26	287	9	86	209	41	47	95	50	106	143	74	25	27	93	30	32	94	152	66.19	6

D = number dead      T = total exposed      % = percentage mortality

\* = previously used papers

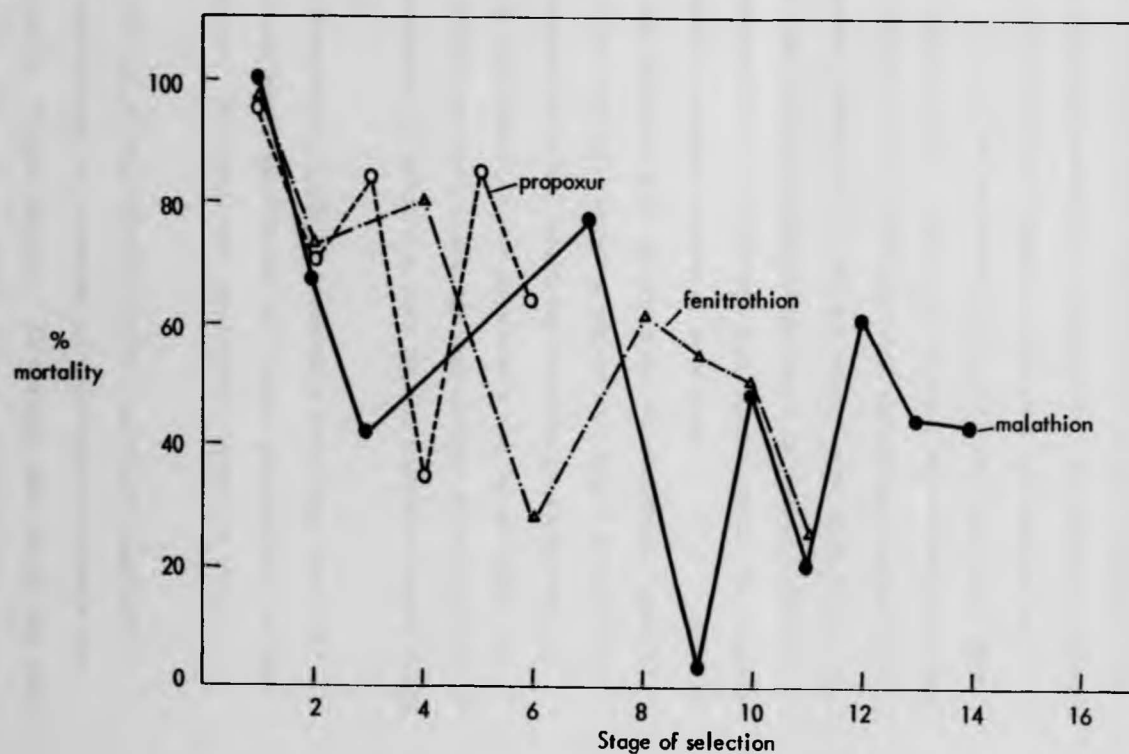
Table 14. Percentage mortalities (numbers tested in parentheses) in variously selected populations of *A. stephensi* from Bangalore, India (ST/BAR) and Iran (ST/ROK) after exposure to the discriminating dosages of malathion, fenitrothion, and propoxur\*

Stage of selection	<u>ST/BAR</u>			<u>ST/ROK</u>	
	malathion selection	fenitrothion selection	propoxur selection	fenitrothion selection	propoxur selection
1	99 (301)	98 (167)	96 (36)	100	100
2	67 (117)	73 (162)	70 (147)	92 (104)	80 (133)
3	42 (132)	-	84 (147)	96 (166)	96 (181)
4	-	80 (25)	34 (47)	93 (45)	17 (48)
5	-	-	85 (455)	69 (90)	54 (46)
6	-	28 (60)	63 (103)		17 (48)
7	77 (245)	-	-		100 (15)
8	-	62 (165)	-	-	0 (35)
9	3 (267)	55 (238)	-	-	66 (131)
10	48 (375)	50 (103)	-	-	
11	20 (61)	25 (69)	-	-	
12	61 (158)	-	-	-	
13	44 (121)	-	-	-	
14	43 (58)	-	-	-	

\* with propoxur the exposure time was 30 minutes

Fig.9

Mortalities in relation to stages in selection in variously selected populations of *A.stephensi* from Bangalore (ST/BAR) after exposure to the discriminating dosages of malathion, fenitrothion and propoxur.



In each of the first five generations, only a sample of the population to be selected was exposed to 5.0% malathion for 15 minutes. The survivors were returned for mating with the unexposed stock population. In each of the next 5 generations on the other hand, the entire population was treated with the same dosage. This process of initially exposing only a proportion, followed by exposure of the entire population was repeated, gradually increasing the exposure time. In fact it involved 30 minutes, one hour and 2 hour exposures to 5.0% malathion. Each step of such selections were made for at least 5 generations. Initially when selecting, particularly when the dosage was increased to one and then 2 hour exposures with 5.0% malathion, the survivors were often small in number. This necessitated generations of unselected maintenance until the density was re-established to enable continued selection.

It was expected that by exposures to sublethal doses of insecticides at the initial stages of selection, rapid elimination of background genetic material which may eventually contribute to stabilisation of resistance in the population may be avoided. In addition, the partial selection adopted was assumed to simulate to some extent the nature of selection that may be occurring under field conditions.

The population SFR/M/17 eventually resulting from this selection, was maintained unselected for 3 more generations yielding the SM35 population. The selection procedure adopted is shown in fig. 10.

The trends in the log-time probit mortality regression lines at the later stages of selection of malathion resistance are represented in Table 15 and fig. 11. It appears that after the rapid initial response, continued selection had not enhanced the resistance

selection for malathion resistance

D = number dead      T = total exposed      % = percentage mortality

Fig.10

Selection of ST/ROK for Malathion resistance.

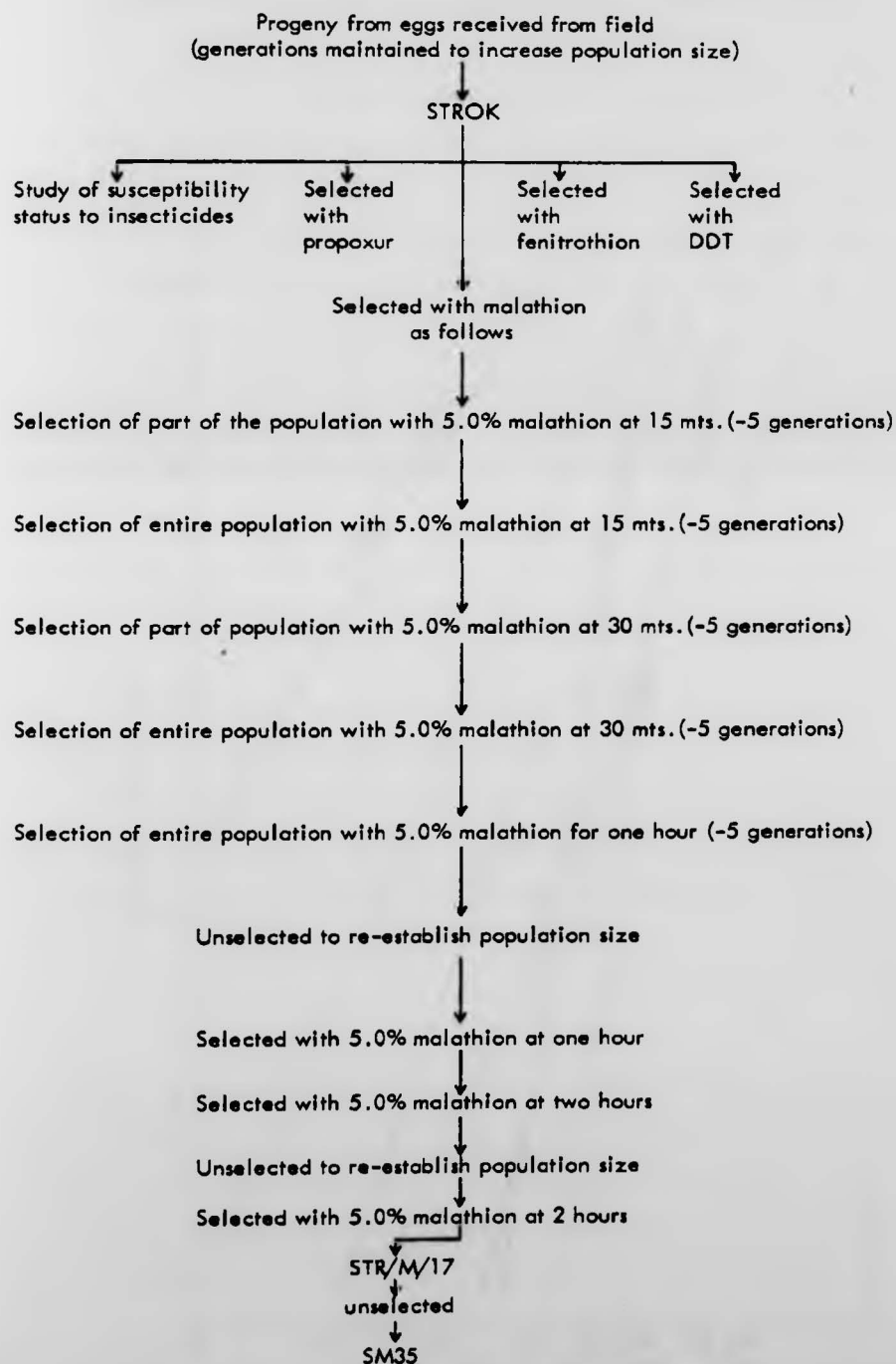
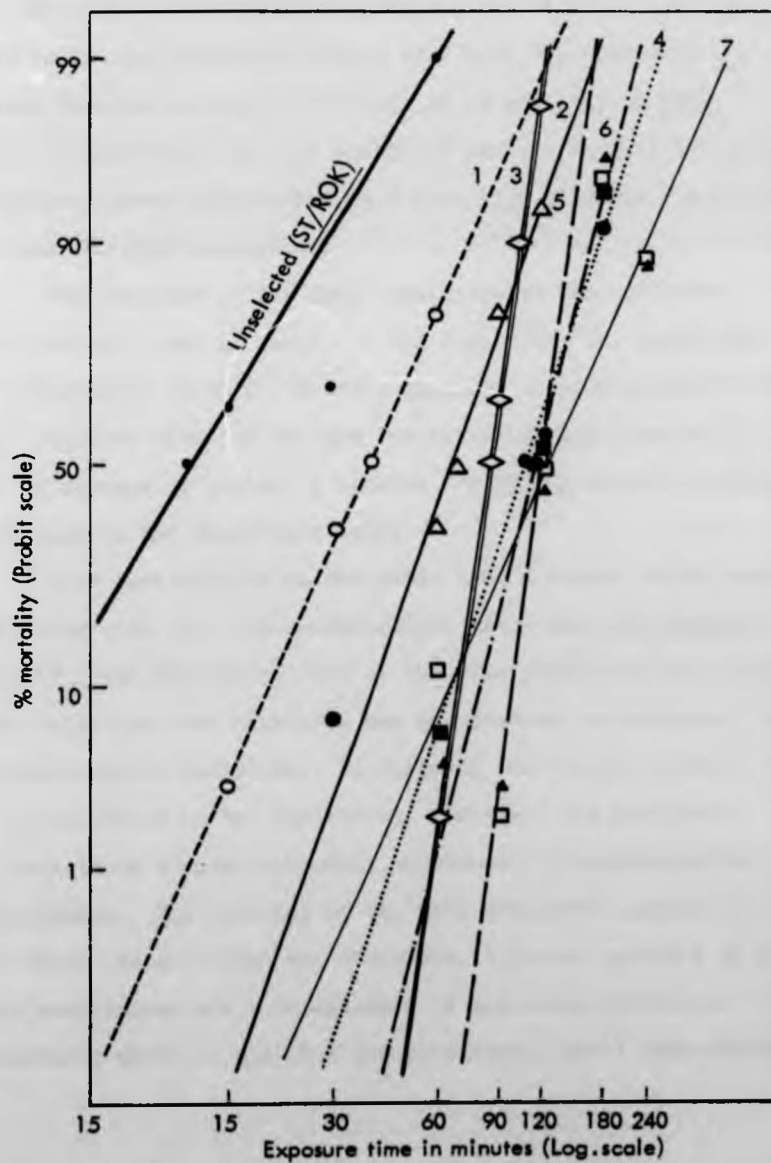


Fig.11

Changes in log time-probit mortality regression lines in *A. stephensi* (ST/ROK) from Iran selected with malathion in the laboratory (stages of selection)





level. The limited attempts to establish a colony from the few survivors of 3 and 4 hours of exposure to 5.0% malathion did not succeed.

The effect of malathion selection on compounds both related and unrelated to it was established through study of the resistance spectrum of the malathion selected SM35 population. In addition, an attempt was made to ascertain the possible detoxication mechanisms involved in the malathion resistance both from the nature of the resistance spectrum as well as the use of synergists, on SM35. The mode of inheritance and the number of genetic factors imparting the malathion resistance was studied on a line E136 selected for homozygosity from the SM35 population.

The response of the SM35 population to the different insecticides are shown in Table 16 and fig. 13A. The population showed a mortality of only 11% after exposure to 5.0% malathion for one hour compared with 99% for the unselected ST/ROK population. The  $LT_{50}$  of 95 minutes as against 9 minutes for ST/ROK showed an eleven-fold increase in the resistance level.

With fenitrothion on the other hand a sample of 802 gave a 97% mortality with the 1.0% concentration after one hour exposure. Here the  $LT_{50}$  was similar to that of the wild population indicating that the selection with malathion had not produced a concurrent increase in the fenitrothion resistance, in spite of the few survivors to the latter in existence in the population. Instead, the population showed resistance towards malaoxon, phenthoate, pirimiphos-methyl and iodofenphos. The response of the wild unselected population to most of these was not known and therefore it is not possible to say if these resistances are a consequence of malathion selection. However, the resistance shown to malaoxon and phenthoate, apart from malathion,

all compounds with carboxyester bonds, may suggest an involvement of carboxyesterase enzymes in these resistances, probably being selected by malathion pressure.

Pretreatment of the SM35 population with TPP showed evidence of synergism with malathion, further supporting the carboxyesterase involvement in this resistance (Table 17, fig. 14). The  $LT_{50}$  of 90 minutes of malathion was reduced by TPP to 23 minutes giving a synergistic ratio of 3.9. DEF, on the other hand, also known to inhibit carboxyesterases in certain instances as well as hydrolytic esterases, showed no effect ( $SR = 0.80$ ) fig. 15. With piperonyl butoxide there was only antagonism ( $SR = 0.53$ ) at all the dosages used (Table 17 fig. 14), in contrast to the observations made on the multiresistant A. culicifacies (CUL/IND) (fig. 4) and the A. albimanus (FERN/RR) strains (fig. 25). The  $LT_{50}$  of 90 minutes obtained with SM35 exposed to malathion alone was increased to 171 minutes when prior exposure was made with PB. This was presumed to be caused by inhibition of mfo's involved in the activation of malathion and thus reduced the amount of toxic malaoxon formed. Prior treatment with a control oil base instead of the synergist showed no obvious effect on the action of malathion (fig. 15). Thus pretreatments do not seem to have effects either on the rate of penetration of insecticides or any other effects of using 2 consecutive treatments.

The probability that malathion pressure alone (as in SM35), had selected only the carboxyesterase detoxication mechanism was thus suggested from a combination of cross resistance, synergistic and probably genetic evidence which will be discussed later. Further that this mechanism did not impart cross resistance to fenitrothion was also demonstrated. However, the few survivors from the discriminating dosage of the latter indicated the existence of its genetic potential

Table 16. Results of exposures of malathion selected SM35 population of *A. stephensi* from Iran  
to various insecticides for varying times

Insecticides	Exposure time in minutes															LT <sub>50</sub> in mins	$\chi^2$	DF																					
	3.0			7.5			15			30			45						60			90			120			180			240			360			480		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%
Chlorphoxim 4.0%	-	-	-	51	168	30	39	112	35	51	102	50	202	287	70	-	-	-	172	180	96	-	-	-	157	164	96	-	-	-	-	-	-	33	26.01	4			
phoxia 2.5%	16	83	19	28	91	31	46	106	43	97	101	96	91	94	97	186	186	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	51.48	4			
Iodofenphos 10.0%	-	-	-	1	76	1	6	111	5	25	144	17	24	99	24	64	104	62	125	180	69	-	-	84	91	92	79	80	99	-	-	-	-	-	85	6.02	6		
pirimiphos methyl 1.0%	-	-	-	-	-	-	-	-	-	142	216	66	87	159	55	-	-	-	201	270	74	-	-	-	-	-	-	-	-	-	-	-	-	20	9.20	1			
fenthion 2.5%	27	125	22	40	96	42	216	236	92	104	104	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	20.57	2				
Fenitrothion 1.0%	-	-	-	226	389	58	26	56	78	187	228	82	776	802	97	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	29.02	2				
phenothate 10.0%	-	-	-	-	-	-	19	100	19	28	184	15	174	300	58	-	-	-	112	177	63	-	-	91	93	98	-	-	-	-	-	-	-	68	61.77	3			
malathion 5.0%	-	-	-	-	-	-	0	39	0	15	221	7	65	616	11	74	84	88	277	406	68	-	-	151	158	96	-	-	-	-	-	-	95	81.30	4				
malaoxon 5.0%	-	-	-	8	400	2	26	586	4	59	376	16	208	519	40	74	169	44	145	191	76	-	-	-	-	-	-	-	-	-	-	-	81	46.20	4				
proposur 0.1%	2	77	3	26	76	36	65	72	90	107	109	98	102	102	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	3.06	3				
permethrin 0.2%	-	-	-	29	81	36	37	226	16	88	172	51	163	280	58	213	253	84	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29	78.57	3				
decamethrin 0.001%	-	-	-	10	76	13	36	99	36	55	83	66	53	90	59	133	182	73	-	-	-	-	159	188	89	-	-	-	-	-	-	-	26	8.69	4				
DOP 4.0%	-	-	-	-	-	-	-	-	-	-	-	-	25	171	15	-	-	-	26	87	30	-	-	84	162	52	-	-	-	56	93	71	231	0.06	2				

D = number dead

T = total exposed

% = percentage mortality

Fig.12

Resistance spectrum of the susceptible population ST15 of A.stephensi from Delhi, India.

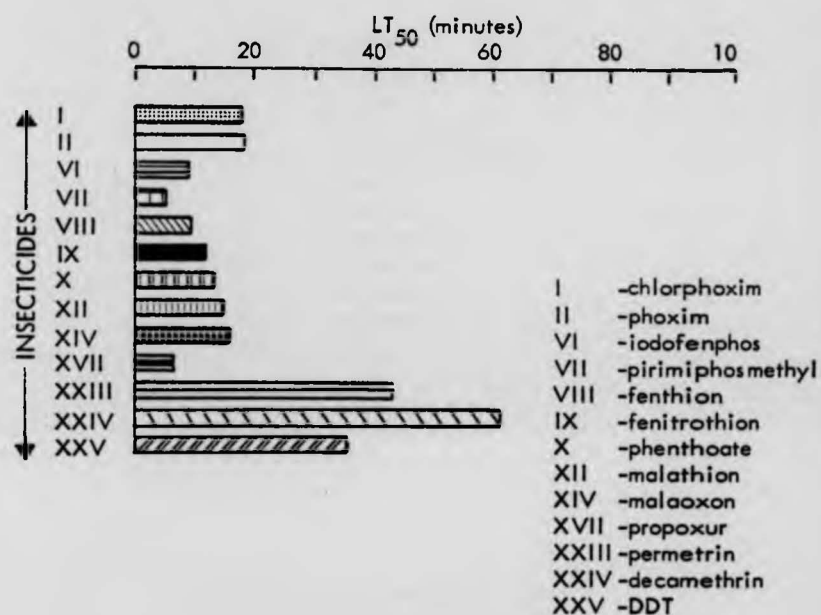


Fig.13 A

Resistance spectrum of the malathion selected population SM35 of A. stephensi from Iran.

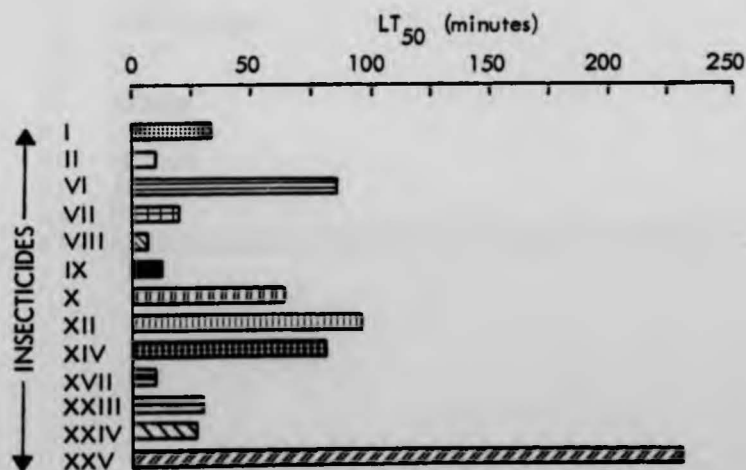


Fig. 13B

Resistance spectrum of the ST/BAR strain of A.stephensi from Bangalore, India.

# INSECTICIDES

I	-chlorphoxim
II	-phoxim
VI	-iodofenphos
VII	-pirimiphos-methyl
VIII	-fenthion
IX	-fenitrothion
X	-phenthoate
XII	-malathion
XIV	-malaoxon
XVII	-propoxur
XXV	-DDT

ST/BAR (from Bangalore, India)

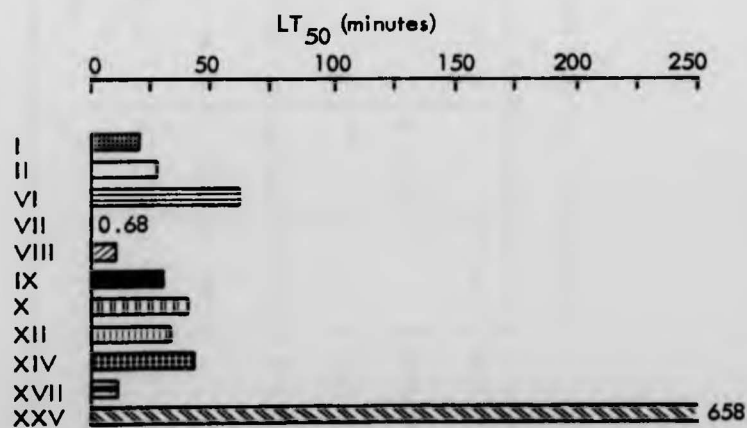


Table 17. Results of exposures of SM35, malathion selected population of *A. stephensi* from Iran,  
to malathion alone and to malathion following pre-treatments with TPP, PB, DEF and control

Compounds and concentration	Exposure time in minutes																								LT <sub>50</sub> in mins	$\chi^2$	SR
	15			30			45			60			90			120			240								
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%						
malathion 5.0%	0	120	0	3	95	3	19	198	10	99	562	18	35	70	50	292	391	75	100	104	96	90.12	8.93	-			
TPP + malathion 5.0%	19	111	17	59	86	69	102	105	97	69	72	96	-	-	-	103	104	99	-	-	-	23.13	-	3.90			
PB + malathion 5.0%	-	-	-	-	-	-	-	-	-	4	96	4	-	-	-	0	106	0	91	98	93	170.7	64.5	0.53			
DEF + malathion 5.0%	-	-	-	0	52	0	-	-	-	2	95	2	15	65	23	-	-	-	-	-	-	113.3	0.06	0.80			
control + malathion 5.0%	1	50	2	-	-	-	22	118	19	9	87	10	-	-	-	96	142	68	-	-	-	94.57	17.28	0.95			

D = number dead    T = total exposed    % = percentage mortality

Fig.14

Mortality relationship in *A.stephensi* (malathion selected SM35 strain) exposed to malathion alone and to malathion after previous exposure to the synergists TPP, and PB.

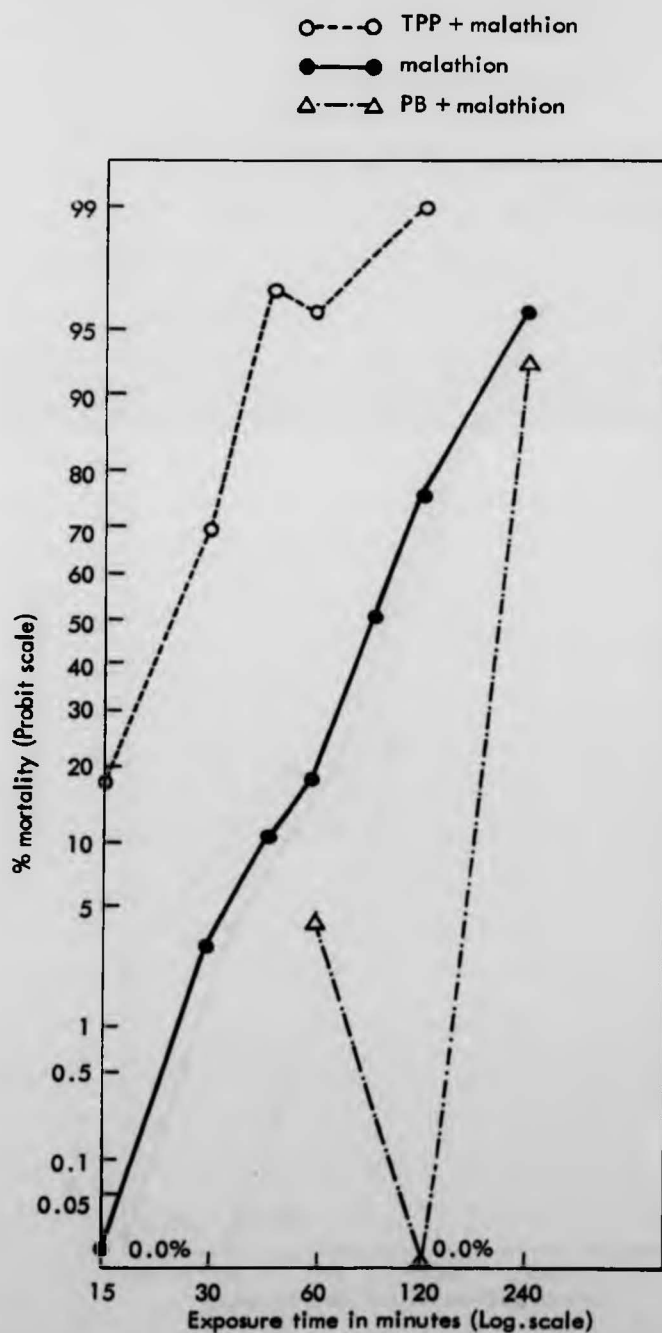
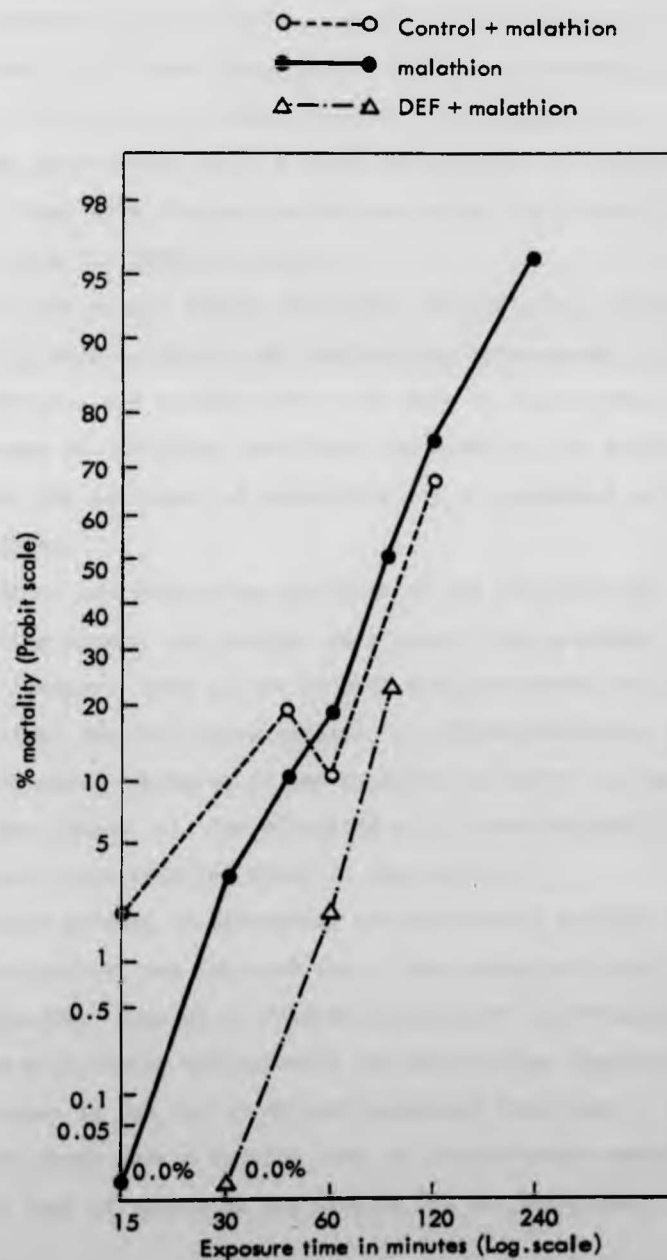


Fig.15

Mortality relationship in *A.stephensi*, (malathion selected SM35 strain) exposed to malathion alone and to malathion after previous exposure to the synergists DEF, and to the Control.





in the population. Therefore attempts were made to determine the effect of fenitrothion selection pressure on the already malathion resistant SM35 population.

Here the few survivors encountered on exposure of SM35 to 1.0% fenitrothion for one hour were inbred. The selection of these was continued at the same dosage either in mass or through single family selection. In the former instance, the selection was followed through many generations until a sufficient number of survivors was obtained. These were then maintained unselected for 3 more generations producing the SMB6 population.

In the single family selection, two families, one with resistance to both malathion and fenitrothion, preferably in a homozygous condition, and another with resistance to fenitrothion only in the absence of malathion resistance was aimed at, in spite of the awareness of the low level of susceptibility to malathion in the SM35 population.

After inbreeding the survivors of the fenitrothion discriminating dosage, egg batches were reared from a number of individual females. Some of the progeny from each were exposed to 5.0% malathion, the rest being exposed to 1.0% fenitrothion, for one hour in each case. Wherever it was possible to derive two egg batches from the same female, all the offspring of one were exposed to malathion and those from the other to fenitrothion.

This process of sib-mating (brother-sister mating) accompanied by selection, was followed for a large number of families for many generations. However no success was achieved in obtaining populations with either homozygosity for fenitrothion resistance or with resistance to the latter without malathion resistance. Instead, a population which gave a certain level of fenitrothion resistance in addition to that of malathion was singled out and maintained

unselected over a number of generations as EM3.16.

Similarly SME<sub>1</sub> and SME<sub>2</sub> were each progeny of an egg batch obtained from a single female. Exposure of all adults of SME<sub>1</sub> to 5.0% malathion for one hour gave no mortality at all while those from SME<sub>2</sub> showed a 58% mortality with 1.0% fenitrothion also at one hour exposure. From SME<sub>1</sub> a line was then selected following exposures to malathion for 3 successive generations of sibmating and sibselection. Complete resistance to malathion treatment was repeatedly encountered. The progeny from 8 such single families were then pooled to give the E136 population. This was continuously maintained under malathion pressure through many generations to ensure full homozygosity. The selection procedure adopted is represented in fig. 16.

This E136 population was used for a study of the mode of inheritance of malathion resistance as well as that of the resistance spectrum.

The populations E136, SMB6, EM3.16 all selected directly or indirectly for a certain level of fenitrothion resistance, the SM35 selected only for malathion resistance and the susceptible ST/15 population, were all compared for their response to the insecticides malathion, fenitrothion, chlorphoxim, phoxim and pirimiphos-methyl. (Tables 11, 16, 18-20) As the selection for fenitrothion resistance was carried out only to a limited extent and therefore the different populations studied were in various stages of heterogeneity, a statistical analysis of the impact of this selection on tolerance to other insecticides is not attempted. However Table 21 compares the different populations both in terms of mortalities at discriminating dosages as well as the  $LT_{50}$  values. In contrast to both the susceptible ST/15, and the SM35 populations, the 3 lines SMB6, EM3.16 and E136 all with increased levels of fenitrothion resistance, appeared

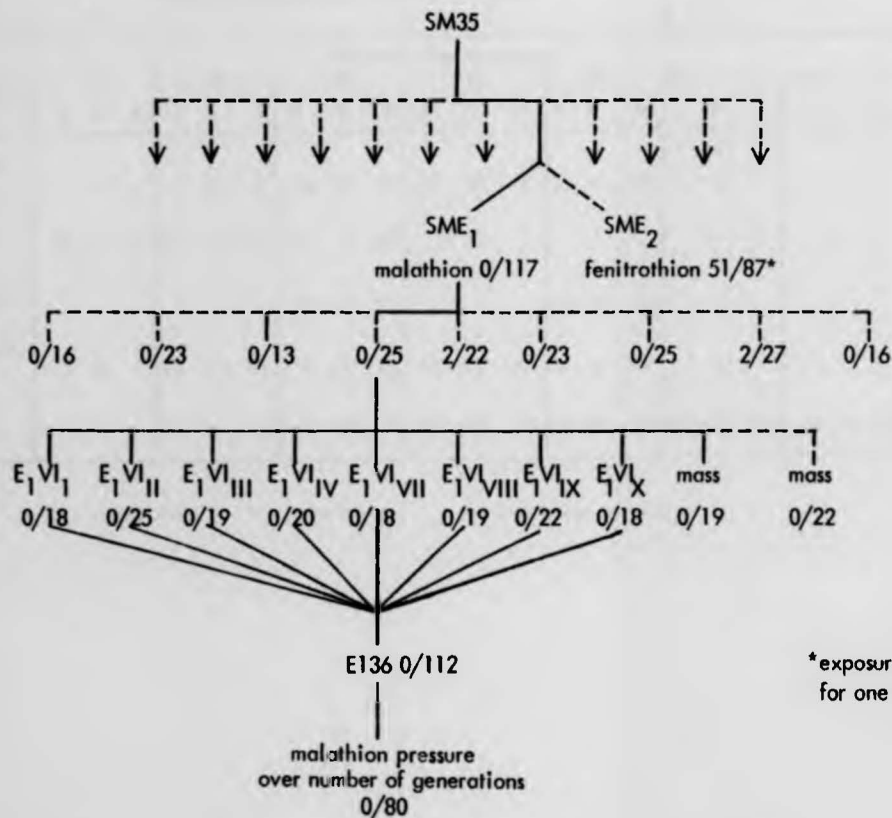
to show a corresponding increase in resistance towards chlorphoxim, phoxim and pirimiphos-methyl (fig. 17). This may therefore be attributed to fenitrothion selection. It is possible that a common factor may be imparting cross resistance to these insecticides, apart from the malathion specific carboxyesterase mechanism already in existence in the malathion resistant individuals. It is also apparent that the level of resistance towards malathion had increased in the malathion-fenitrothion selected lines more than in the SM35 line selected only with malathion. It is not known if this enhanced effect is due to cross resistance also imparted to malathion by the common factor already suggested for other insecticides or if the expression of the carboxyesterase mechanism for malathion is more effective in the presence of other resistance factors. It may also be possible that if a wider range of insecticides was examined, a broader resistance spectrum would have been encountered.

The potential for the development of resistance towards fenitrothion and therefore probably through cross resistance to the other insecticides, in the parent ST/ROK population was also indicated through the selection of the survivors of the discriminating dosages of fenitrothion. Here the 100% mortality in ST/ROK was reduced to 69% in the selected sample on exposure to 1.0% fenitrothion for one hour. Similarly, the existence of the potential for the development of propoxur resistance has also been indicated through selection with this insecticide (Table 14 , fig. 18). The selection initiated with a 15 minute exposure to the 0.1% of this insecticide was subsequently continued at a 30 minute exposure on the same concentration. The 100% mortality at 15 minutes exposure in the parent ST/ROK was reduced to 66% after a 30 minute exposure to 0.1% concentration.

Comparison of the response of the two sexes was made in

Fig.16

Single family selection for malathion resistance for genetic studies in *A. stephensi* (figures represent exposures to 5.0% malathion at one hour-number dead/ total exposed).



\*exposure to 1.0% fenitrothion for one hour(number dead/total tested)

Table 18 Results of exposures of malathion selected E146 population (but also containing some fenitrothion resistance) of *A. stephensi* from Iran to various insecticides for varying times

Insecticides	Exposure time in minutes																		LT <sub>50</sub>	χ <sup>2</sup>	DF				
	30			45			60			90			120			180						240			
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%	
chlorphoxim 4.0%	-			7	76	9	60	171	35	46	67	69	32	32	100	-			-				70	4.69	2
phoxim 2.5%	15	59	25	80	99	81	47	54	87	-			-			-			-				36	5.85	1
pirimiphos methyl 1.0%	-			-			26	137	19	-			24	38	63	-			-						
fenitrothion 1.0%	3	41	7	24	111	22	218	276	79	169	187	90	36	36	100	-			-				51	33.79	3
malathion 5.0%	-			-			0	80	0	66	202	33	38	187	20	106	106	100	73	74	99	124	100.76	3	

D = number dead      T = total exposed      % = percentage mortality

Table 19. Results of exposures of malathion-fenitrothion selected SMR6 population of *A. stephensi* from Iran to various insecticides for varying times

Insecticides	Exposure time in minutes															LT <sub>50</sub> in minutes	χ <sup>2</sup>	DF						
	30			45			60			90			120						180			240		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%	D	T	%
chlorphoxim 4.0%	7	92	8	23	71	32	83	116	76	90	91	99	126	127	99	-	-	-	50	3.64	3			
phoxim 2.5%	36	120	30	117	151	78	122	126	97	-	-	-	29	109	27	-	-	-	256	184.54	2			
pirimiphos methyl 1.0%	6	133	5	50	149	34	24	86	28	-	-	-	125	134	93	-	-	-	63	19.87	2			
fenitrothion 1.0%	28	125	22	125	223	56	195	245	80	27	27	100	88	89	99	-	-	-	42	17.09	4			
malathion 5.0%	-	-	-	1	75	1	6	96	6	5	43	12	136	208	65	92	92	100	74	75	99	106	-	-

D = number dead T = total exposed % = percentage mortality

Table 20. Results of exposures of malathion-fenitrothion selected EM 3.16 population of *A. stenohensi* from Iran to various insecticides for varying times

Insecticides	Exposure time in minutes																		LT <sub>50</sub>	χ <sup>2</sup>	DF			
	30			45			60			90			120			180						240		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%
chlorphoxim 4.0%	0	16	0	20	108	19	46	166	30	-	-	-	59	69	86	-	-	-	-	-	-	75	3.65	2
phoxim 2.5%	29	79	37	53	87	61	80	88	91	-	-	-	-	-	-	-	-	-	-	-	-	36	3.88	1
pirimiphos methyl 1.0%	-	-	-	22	114	19	12	79	15	-	-	-	49	101	49	-	-	-	86	86	100	98	25.11	1
fenitrothion 1.0%	8	58	14	33	68	49	176	213	83	131	153	86	36	37	97	-	-	-	-	-	-	44	21.45	3
malathion 5.0%	0	16	0	-	-	-	2	117	2	-	-	-	58	104	56	120	124	97	44	44	100	113	1.46	3

D = number dead    T = total exposed    % = percentage mortality

Table 21. Percentage mortalities at one hour exposure to various insecticides and  $LT_{50}$  values of the different populations of *A. stephensi*: the malathion selected SM35, the malathion-fenitrothion selected SMB6, EM3.16 and E136 derived from ST/ROK from Iran, and the susceptible ST/15 from Delhi, India.

	susceptible malathion selected		malathion-fenitrothion selected		
	ST/15	SM35	SMB6	EM3.16	E136
malathion	98	11	6	2	0
fenitrothion	100	97	80	83	79
chlorphoxim	95	70	76	30	35
phoxim	98	100	97	91	87
pirimiphos-methyl	92	55	28	15	19
(LT <sub>50</sub> )					
malathion	15	95	106	113	124
fenitrothion	12	13	42	44	51
chlorphoxim	18	33	50	75	70
phoxim	19	10	30-45	36	36
pirimiphos-methyl	5	20	63	98	-



Fig.17

A comparison of the  $LT_{50}$  values for a number of insecticides in five populations of *A.stephensi* to show the effect of selection with malathion, from that of malathion followed by fenitrothion.

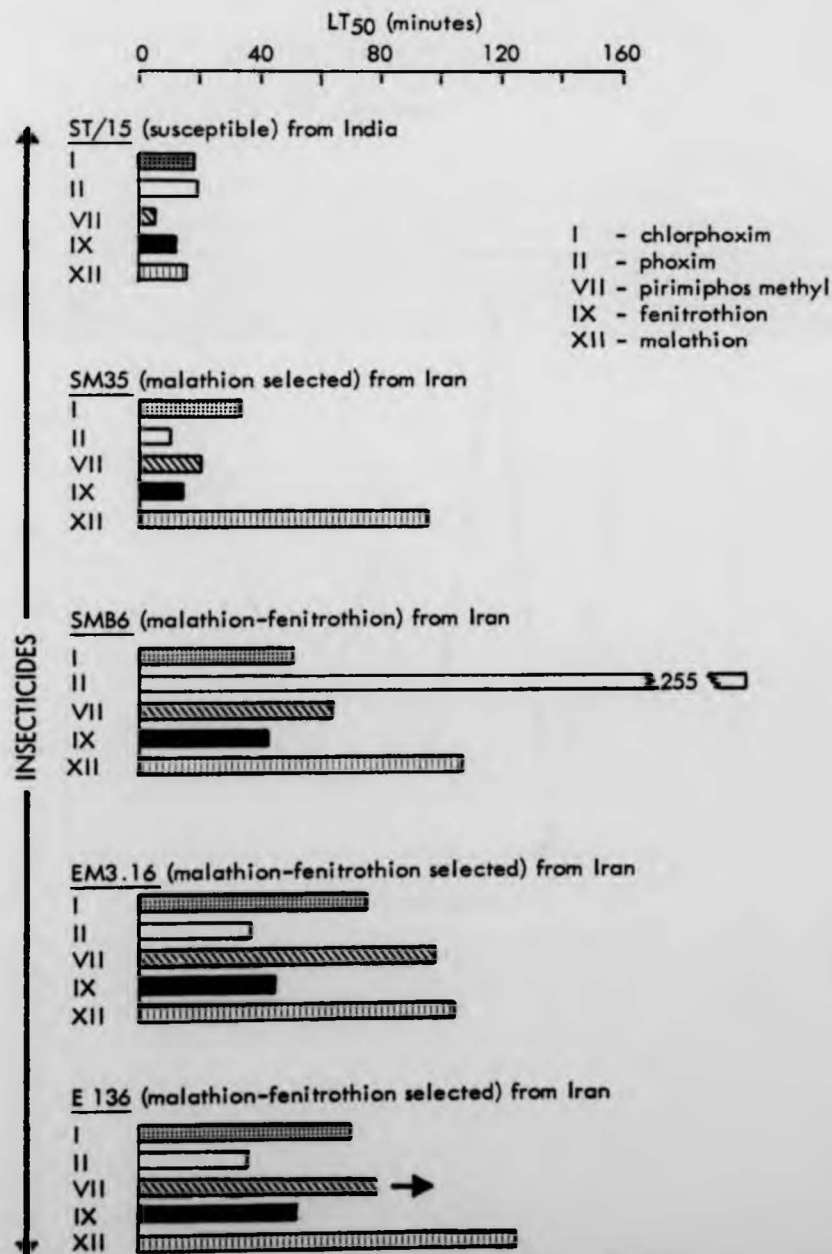


Fig.18

Mortalities in relation to stages in selection of *A.stephensi* from Iran (ST/ROK) with fenitrothion and propoxur after exposure to the discriminating dosages of these insecticides.

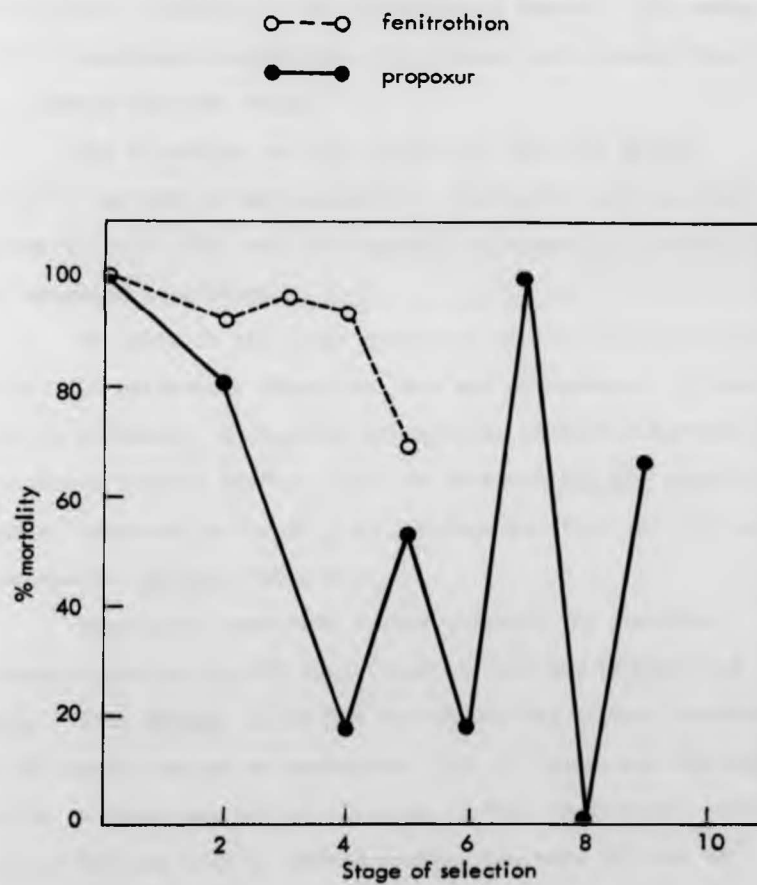
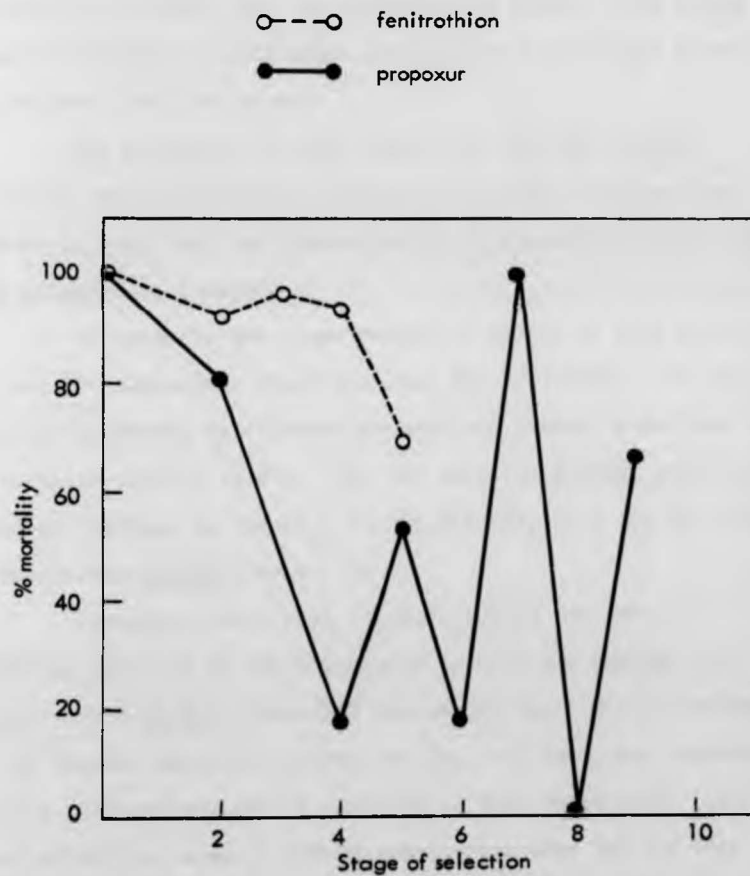


Fig.18

Mortalities in relation to stages in selection of *A.stephensi* from Iran (ST/ROK) with fenitrothion and propoxur after exposure to the discriminating dosages of these insecticides.



the different population of A. stephensi towards malathion, fenitrothion, chlophoxim, phoxim, and pirimiphos-methyl. The two sexes showed no difference in their response to malathion, whether in the susceptible or the resistant populations. The ST/15 and ST/BAR populations also showed no difference among the sexes in their susceptibility towards all the insecticides tested. But among the different resistant populations, the females were always found to be more tolerant than the males.

The selection for DDT resistance from the ST/ROK population was made by mass selection, initially with one hour exposure to 4.0% DDT, and subsequently increased to 2 hours and later extended to 4 hours.

In spite of the large number of adults of both sexes which survived DDT selection, there was poor egg production. It was therefore necessary to maintain generations without selection to re-establish density levels. The DDT selected STR/DDT population showed an increase in the  $LT_{50}$  to 356 minutes, from the 152 minutes in the unselected ST/ROK (Table 22).

Synergists were used to characterise the possible mechanisms involved in DDT resistance in both the STR/DDT and ST/BAR. With ST/BAR, where DDT resistance was higher, pretreatment with PB clearly showed no synergism (fig. 18) which may indicate that mixed function oxidases may not be involved in this resistance. The  $LT_{50}$  values of DDT and that of DDT-PB combination were 658 and 660 minutes respectively (SR = 1.0). With F-DMC on the other hand the  $LT_{50}$  of 658 minutes with DDT alone was reduced to 290 minutes, the synergistic ratio being 2.3, thus indicating the importance of DDT-ase in this resistance (Table 23).

With STR/DDT, pretreatments with both PB and sesamex showed no consistent effect on its toxicity as seen in the figs. 19 and 21. The SR calculated for sesamex and DDT was 0.57 and that with PB was 0.48, showing a net antagonism in both instances. When F-DMC was used the resulting SR of 3.32 indicated the importance of DDT-ase in this Iranian population also. However, Table 24 fig. 21 shows the combined effect of two synergists F-DMC and PB on DDT, in which the SR of 4.75 obtained exceeds the 3.32 obtained with F-DMC alone. This observation is difficult to interpret and further investigations may be necessary on this aspect.

In the use of synergists by this contact method of administration, it may not be possible to establish if a mechanism concerned could be completely inhibited by the synergist. In the case of DDT-ase, the F-DMC dosage used could not be increased further due to its toxicity. In addition, in the STR/DDT as was also observed in many other species, exposures to DDT was found to cause a knockdown effect on some individuals which may or may not recover. Thus in this population on a sample of 67 adults exposed to 4.0% DDT for one hour 27 were knocked down following exposure while the total deads recorded following a 24 hour holding period was only 12. Similarly although 273 adults were knocked down following an exposure for 4 hours, to DDT, the mortality recorded following a holding period was only 148. It was not established the extent to which the sample knocked down is represented among the dead. However, it is obvious that at least a certain proportion of these have eventually recovered. It is therefore possible to assume that the quantities of either the synergist or the insecticide picked up by the individual mosquitoes may not be comparable in this technique of application. The opportunities for picking up the respective

Table 22. Results of exposures of BDT selected *HTH/BDT* population of *A. stephensi* from Iran to various insecticides for varying times

Insecticides	Exposure time in minutes																								LT <sub>50</sub> in mins	χ <sup>2</sup>	DF			
	15			30			45			60			90			120			240			480						960		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%
chlorphoxin 4.0%	-			-			26	161	16	113	299	38	-			102	182	56	110	111	100	-			-			85	25.47	2
phoxin 2.5%	14	40	35	71	88	81	58	66	88	80	86	93	-			-			-			-			-			18	2.38	2
fenitrothion 1.0%	3	38	8	74	201	37	330	377	88	128	136	94	-			-			-			-			-			32	19.06	2
phenthoate 10.0%	-			-			3	132	2	86	98	88	-			112	177	63	91	93	98	-			-			74	349.26	2
malathion 5.0%	7	142	5	3	171	2	11	241	5	74	364	20	74	153	48	99	200	50	95	95	100	-			-			105	887.23	5
malaoxon 3.0%	5	92	5	15	106	14	60	143	42	48	83	58	74	169	44	145	191	76	-			-			-			70	34.91	4
permethrin 0.2%	89	148	60	246	320	77	96	108	89	299	370	81	69	81	85	52	55	95	-			-			-			7	10.62	4
DDT 4.0%	-			-			-			118	713	17	-			190	1015	19	340	824	41	172	336	51	113	122	93	356	55.91	3

D = number dead      T = total exposed      % = percentage mortality

Table 23. Results of exposures of ST/BAR population of *A. stephensi*, from Bangalore, India to DDT alone, and to DDT following pretreatments with PB and F-IMC

Compounds and concentration	Exposure time in minutes															LT <sub>50</sub>	χ <sup>2</sup>	SR			
	60			120			240			360			480						960		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%
DDT 4.0%	1	126	1	0	135	0	9	179	5	6	64	9	2	69	3	65	70	93	658	4	-
PB + DDT 4.0%	1	44	2	0	55	0	13	82	16	24	154	16	8	33	24	23	25	92	660	38.25	0.996 Or 1.0
FIMC + DDT 4.0%	4	89	4	29	87	33	70	138	51	38	81	47	60	95	63	-			290	13.08	2.30

D = number dead

T = total exposed

% = percentage mortality

Fig.19

The mortality relationship in *A.stephensi* (ST/BAR) exposed to DDT alone and to DDT after previous exposure to F-DMC and PB.

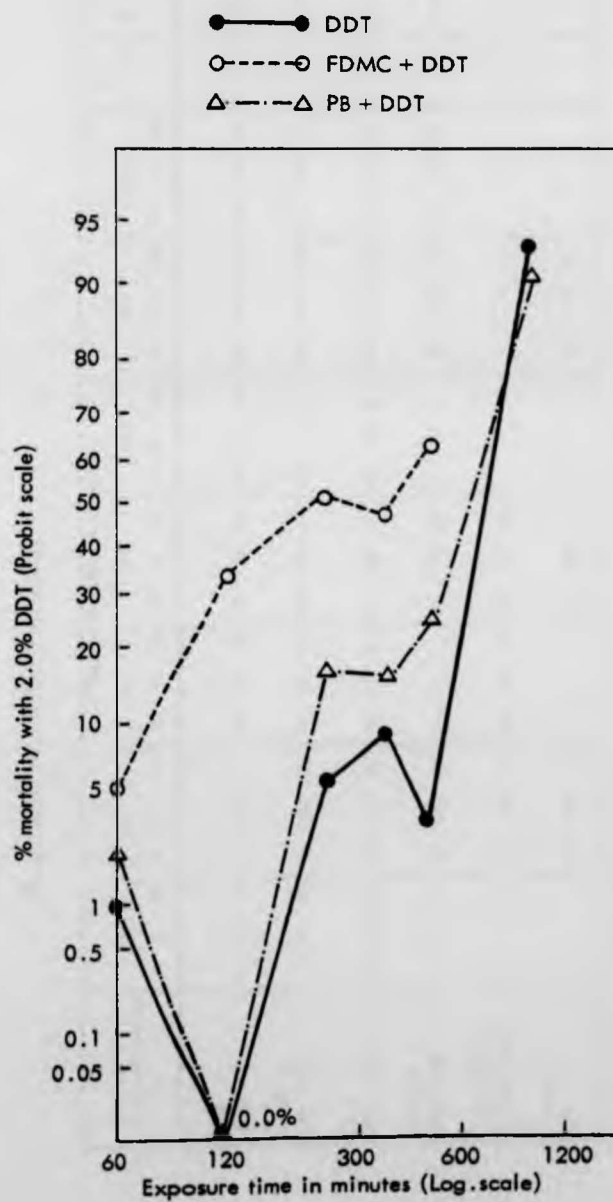




Table 24. Results of exposures of SPB/DDT, DDT selected population of *A. stephensi* from Iran, to DDT alone and to DDT following pretreatments with F-IMC, PB, sesamex, PB + F-IMC, and control

Compounds and concentration	Exposure time in minutes																					LT <sub>50</sub>	χ <sup>2</sup>	SR
	30			60			90			120			180			240			960					
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%			
DDT 4.0%	0	26	0	10	213	5	2	24	8	51	170	30	14	32	44	121	266	46	13	42	31	418	56.77	
F-IMC + DDT 4.0%	6	30	20	76	194	39	18	40	45	86	188	46	23	30	77	163	261	63	62	85	73	126	9.79	3.32
PB + DDT 4.0%	11	39	28	73	361	20	18	40	45	40	350	11	18	24	75	123	322	38	52	101	52	875	72.7	0.48
sesamex + DDT 4.0%	-			26	147	18	-	-		45	115	39	-			50	150	33	43	85	51	819	8.35	0.57
PB + F-IMC + DDT 4.0%	-			9	40	23	28	62	45	26	31	84	-			-			-			88	2.52	4.75
control + DDT	-			20	109	18	-			45	170	27	-			45	100	45	-					

D = number dead      T = total exposed      % = percentage mortality

Fig.20

Mortality relationship in A.stephensi (STR/DDT) exposed to DDT alone and to DDT after previous exposure to the synergist PB.

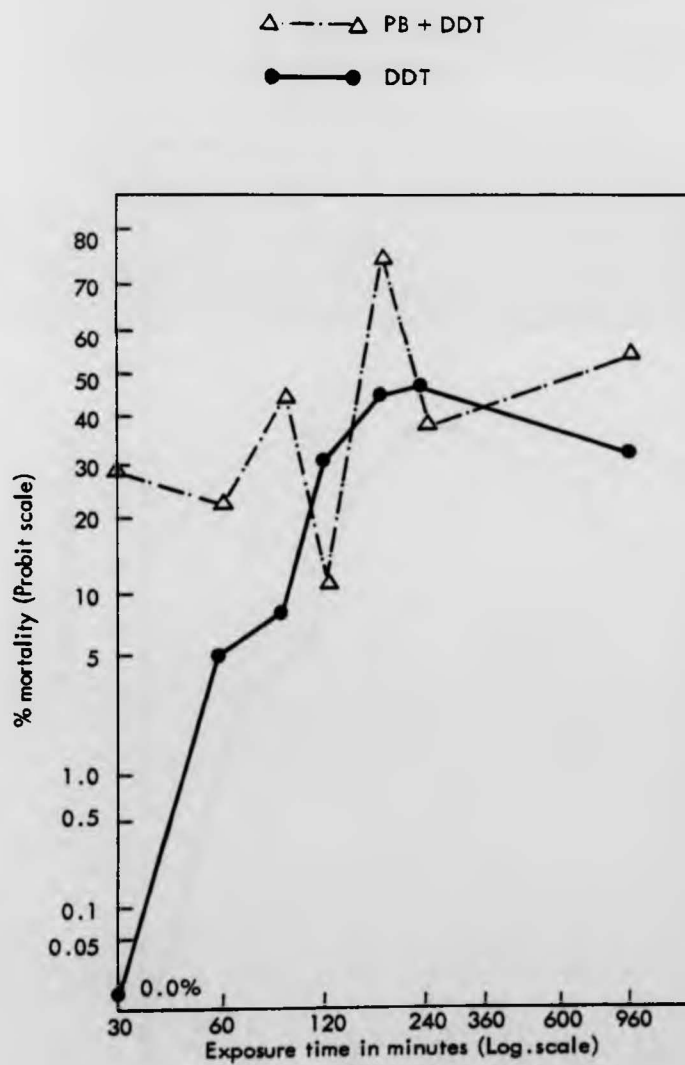


Fig.21

Mortality relationship in *A.stephensi* (STR/DDT) exposed to DDT alone and to DDT after previous exposure to synergists F-DMC, and FDMC + PB.

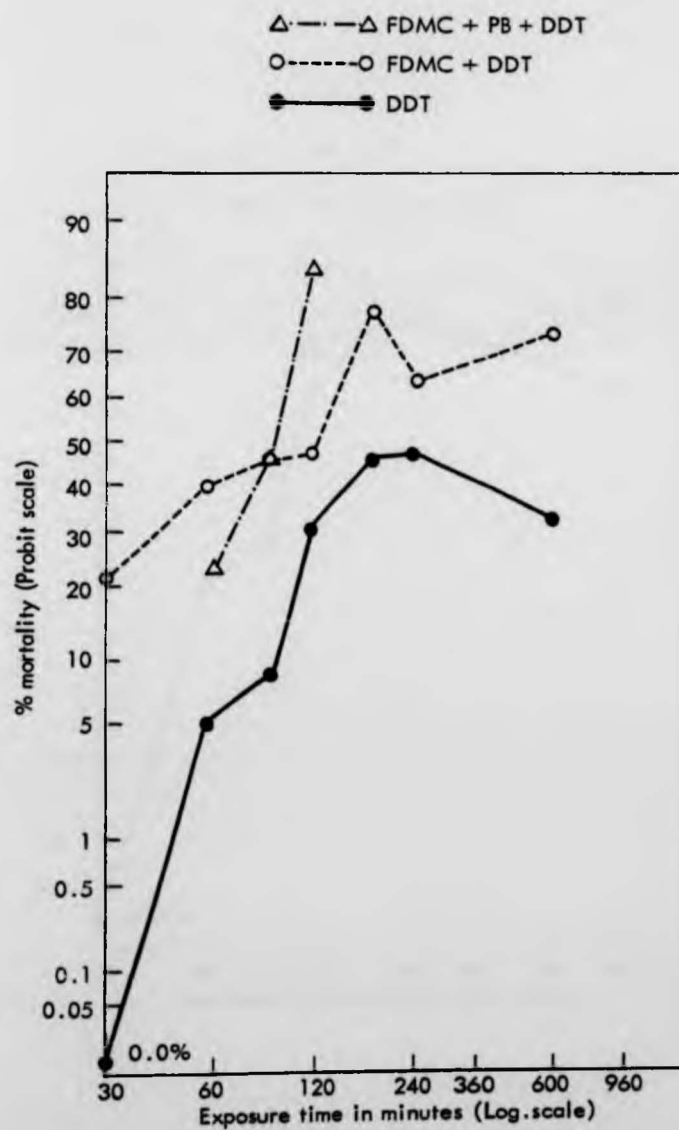


Fig.22

Mortality relationship in *A.stephensi* (STR/DDT) exposed to DDT alone and to DDT after previous exposure to the synergist sesamex.

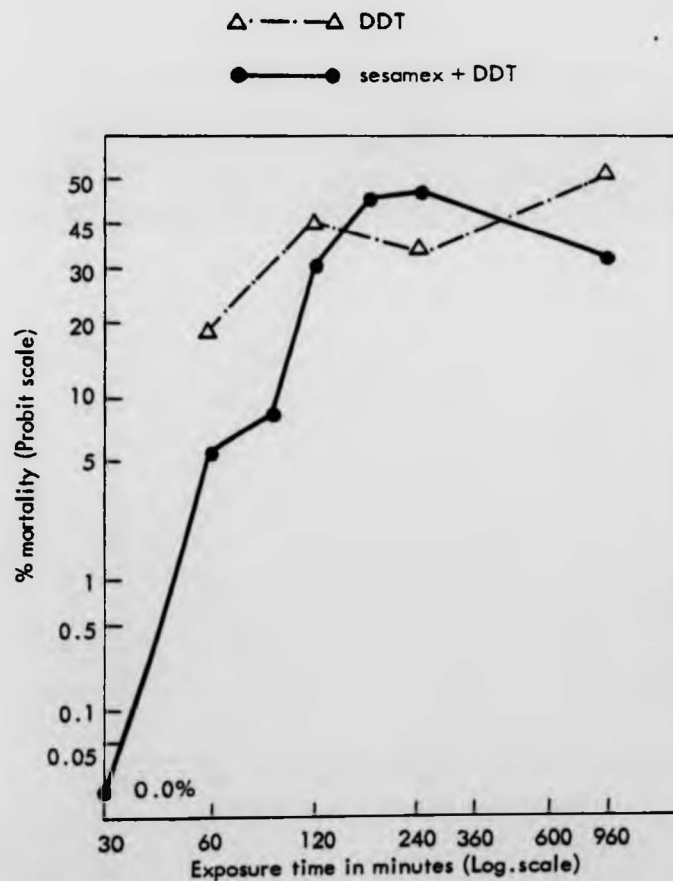
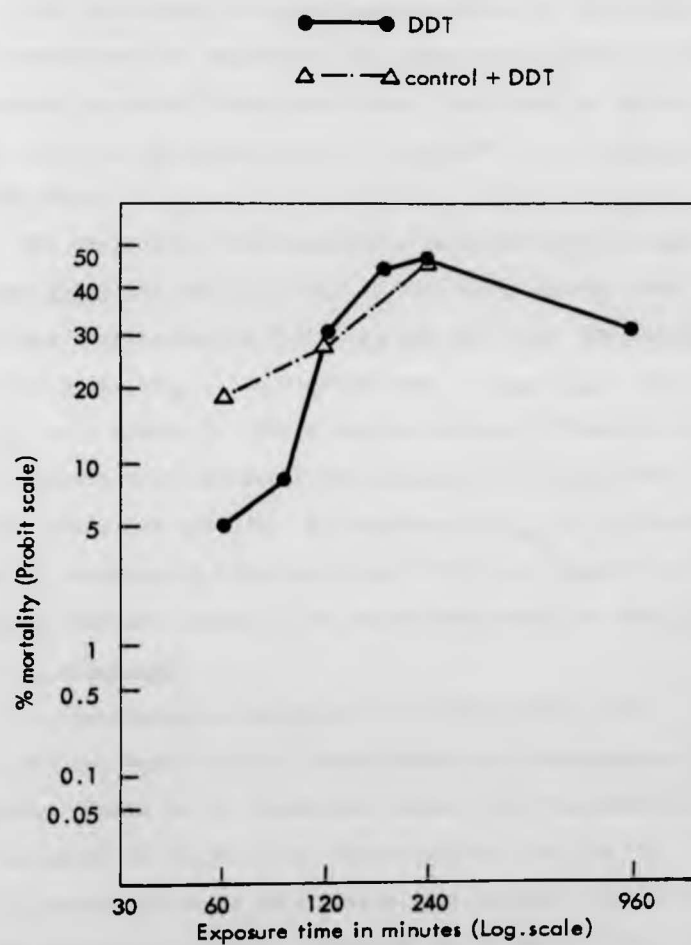


Fig.22a

Mortality relationship in *A.stephensi* (STR/DDT)  
exposed to DDT alone and to DDT after  
previous exposure to control (oil base).



compounds could vary between mosquitoes knocked down and those in continued contact with the compound concerned. Such variations in the quantities picked up could vary even among those not knocked down at all, to a certain extent.

This phenomenon may also have an effect on the extent and speed of development of resistance in populations whether selected in the laboratory or under field conditions. The speed of selection will depend on which of the genotypes, the resistant or the susceptible, are knocked down.

The response of the homozygous resistant E136 strain, the susceptible ST/15 and the  $F_1$  hybrid of the cross between them, to 5.0% malathion are represented in Table 25 and fig. 23. The resistant level of the E136 ( $LT_{50}$  = 124 minutes) was 8 times that of the ST/15 ( $LT_{50}$  = 15 minutes). There was no obvious difference in the responses between the hybrids of the reciprocal matings, the mortalities being 28% and 31%. The estimated  $LT_{50}$  of 72 minutes according to Georghiou's interpretation (1969) was suggestive of an incompletely dominant nature of malathion resistance in the E136 strain of A. stephensi.

In the backcross progenies the dosage used (5.0% malathion for one hour) did not discriminate the susceptibles from the heterozygotes (Table 25). Therefore taking into consideration the average mortality of 29.5% of the heterozygotes, and the 2% survival of susceptibles at this dosage, the expected mortalities in the backcross progenies would be expected to be 98% of  $X/2$  + 29.5% of  $X/2$ , where X is the number tested. This should on an average produce a 64% mortality for monofactorial inheritance.

Table 26 showing the results of exposures of 7 families in the first backcross offspring showed only 2 to be in agreement with

Table 25. Results of exposures of the resistant E136 strain of *A. stephensi* from Iran, the susceptible ST/15 strain from Delhi, and the hybrids between them, to 5.0% malathion for varying periods of time

Period of exposure (minutes)	E136			ST15			F <sub>1</sub> progeny								
							E136 00 x ST15 00						ST15 00 x E136 00		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%
7.5	-			71	297	24	-			-			-		
15	-			318	671	47	-			-			-		
30	-			726	932	78	-			-			-		
45	-			589	622	95	-			-			-		
60	0	80	0	1036	1054	98	31	111	28	37	120	31	37	120	31
90	66	202	33	-			63	78	81	85	103	83	85	103	83
120	38	187	20	-			36	39	92	45	52	87	45	52	87
180	106	106	100	-			42	42	100	30	30	100	30	30	100
240	73	74	99	-			-			-			-		
Lt <sub>50</sub> mins		124			15			72			72			72	
Lt <sub>90</sub> mins		190			37			110			118			118	
slope		6.92			3.13			7.07			6.03			6.03	

D = number dead    T = total tested    % = percentage mortality

Fig.23

Log-time probit mortality regression line for *A.stephensi* from Iran (E136), Delhi, India (ST/15) and hybrids between them exposed to malathion.

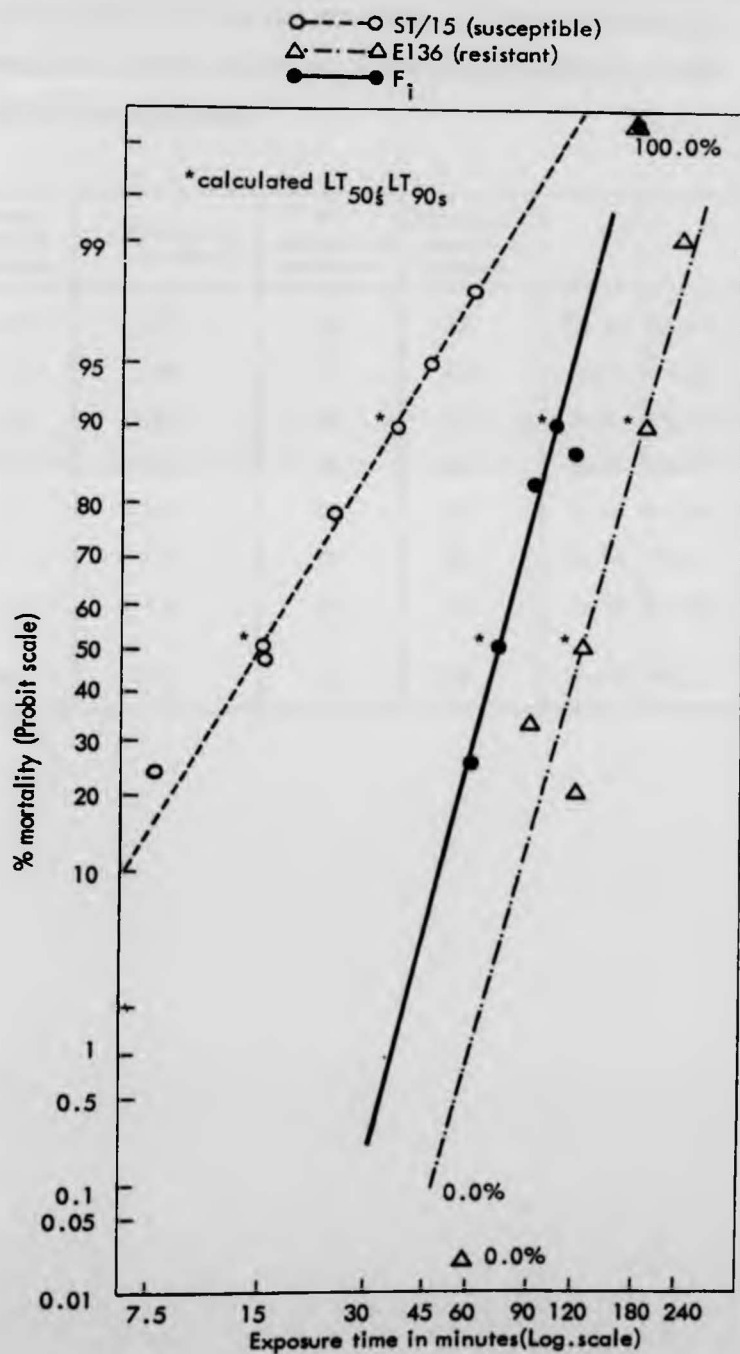




Table 26. Single family results of exposures to 5.0% malathion for one hour, in the offspring of the first backcross of the hybrid (*A. stephensi*)

Batch number	Total number tested	Mortality (number)	% mortality observed	Mortality expected (number)	$\chi^2$	P
1	123	47	38	79	12.96	<0.01
2	63	29	46	40	3.03	>0.05
3	49	40	82	30	2.0	>0.10
4	73	27	37	47	8.51	<0.01
5	55	21	38	35	5.60	<0.05
6	107	37	35	68	14.13	<0.01
7	40	14	35	26	5.54	<0.05
Total	510	215	42	325	37.23	<0.01

Table 27 Single family results of exposures to 5.0% malathion for  
one hour in the second backcross involving resistant E136  
strain and susceptible ST/15 strains of A. stephensi

Batch number	Total number tested	Number dead	% mortality	Mortality expected (number)	$\chi^2$	P
1	320	204	64	204	0.00	-
2	336	205	61	215	0.47	< 0.05
3	186	133	72	118	1.91	> 0.1
4	90	36	40	57	7.74	< 0.01
5	110	56	51	70	2.80	> 0.05
6	105	86	82	67	5.39	< 0.05
7	187	165	88	120	16.87	< 0.01
8	266	134	50	169	7.25	< 0.01
Total	1600	1019	64	1020	0.00	-

Table 28. Summary of the results of exposures of the progenies of three consecutive backcrosses with selection (involving the resistant E136 strain of *A. stephensi* from Iran and the susceptible ST/15 strain from Delhi, India to 5.0% malathion for one hour

	first backcross	second backcross	third backcross
total number tested	510	1600	858
no. susceptible	215	1019	382
% mortality	42	64	45
mortality expected (number)	325	1020	547
$\chi^2$	37.23	0.00	49.77
P	<0.01	-	< 0.01

with this expected mortality. In the second backcross out of 8 families tested 4 were in agreement. However, when the data from all 8 families were pooled there was excellent agreement with the expected value  $\chi^2 = 0.0$  on a sample of 1600 mosquitoes. In the third backcross, where the rearing was carried out in mass, the results showed a significant departure from the 64% mortality expected  $\chi^2 = 49.77$   $P < 0.01$ .

It should be noted however, that in all three backcrosses, the significant departures shown by the high  $\chi^2$  values were all showing a similar trend in that there was a shortage of susceptible phenotypes. In all instances, the mortalities were less than the expected 64%. Why this was so is not known.

However, the fact that there was no tendency for increasing mortalities in the consecutive backcrosses accompanied by selection, (Table 28) favours a monofactorial interpretation of the mode of inheritance of this resistance factor.

#### A. albimanus:-

The susceptibility status of the two populations PALB (from Panama) and FERN/RR (from El Salvador) to a series of insecticides has been compared (Tables 29 and 30 and fig. 24). PALB showed high susceptibility to most insecticides. With decamethrin 0.001% however, a two hour exposure was necessary for a complete kill. There were survivors from exposures to 5.0% malaoxon both at one and two hours in spite of high susceptibility towards all other OP's. The possibility of this being an effect of the age of the insecticide papers is considered. The very high susceptibility of this strain towards DDT is evident from the 100% mortality encountered even at a 15 minute exposure to 4.0% DDT.

The high susceptibility of the PALB strain to almost all the insecticides tested may be due to its                      been maintained in the laboratory for a considerable period of time, more than 30 years. It is probable that the samples were collected prior to extensive selection by insecticides in the field.

The multiresistant FERNS/RR population on the other hand showed resistance to all the OP insecticides tested with the exception of fenthion. To this insecticide a high susceptibility was shown. Here a 30 minute exposure to the 2.5% concentration was sufficient for a complete mortality. Irrespective of the fact that this population had been selected further in the laboratory with propoxur, the level of resistance appeared to have declined from that shown at the time of receipt in 1974. Thus the 3% mortality encountered in 1974, has now increased to 46% after one hour exposure to 0.1% concentration of the insecticide. Similarly the DDT resistance reported as 16% mortality with the 4% concentration on one hour exposure in 1974 has now changed to a 92% mortality on the same dosage, indicating a reversion towards susceptibility. However, a 15 minute exposure gave only a 31% kill, which dosage, as pointed out earlier, killed all of the PALB strain. Of all the populations tested, this species appears to require the lowest exposure time to the discriminating dosage of DDT.

There was no obvious difference in response between the susceptible and resistant strains to the two pyrethroids permethrin and decamethrin.

Synergists were used to characterise the nature of the detoxication mechanisms involved in the malathion resistance shown by the FERNS/RR strain. Ariyaratnam and Georghiou (1971) demonstrated the involvement of carboxyesterases in malathion resistance in the

larvae of A. albimanus through synergism by TPP.

Synergists used in this instance were PB, TPP, DEF and SV<sub>1</sub> (Table 31, fig. 25). The latter is known to inhibit carboxy-esterases as well as oxidases.

TPP produced synergism (SR = 1.5), the LT<sub>50</sub> of 116 minutes for malathion being reduced to 78 minutes following prior treatment with the synergist. This observation in addition to supportive evidence from the resistance to malaaxon and also phenthoate may be suggestive of a carboxyesterase involvement. DEF however produced no obvious synergism (SR = 1.1) as was also the case with A. stephensi SM35 population. Piperonyl butoxide showed evidence of both synergism and antagonism with a net trend towards the former (SR = 1.1). The LT<sub>50</sub> of 116 minutes for malathion was reduced to 106 minutes by PB. For reasons already discussed in the case of A. culicifacies, evidence of synergism even to a slight extent may suggest an inhibition by PB of a mechanism involved in the breakdown of malathion, apart from the inhibitory effect on the activation of this insecticide.

On the two occasions where SV<sub>1</sub> was used, the initial test gave a SR of 1.9, while the repeated test produced a synergistic ratio of 4.0. Both these observations have shown an enhancement of the toxicity of malathion greater than that produced when TPP was used. Therefore, on this evidence it can be presumed that in addition to carboxyesterases, mixed function oxidase system may also be involved in the resistance towards malathion in this population as was also suggested for the multiple resistant A. culicifacies.

Table 79. Results of laboratory exposures of *Anopheles albimanus* (PALE) from Panama to various insecticides for varying times

Insecticides	Exposure time in minutes															LT <sub>50</sub> in minutes	χ <sup>2</sup>	DF									
	3			7.5			15			30			45						60			120					
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%						
chlorphoxia 4.0%	18	56	32	-	-	-	155	166	93	122	126	97	26	29	90	138	138	100	-	-	-	4	-	-	31.56	3	
phoxia 2.5%	2	44	5	-	-	-	46	71	65	171	172	99	59	59	100	-	-	-	-	-	-	10	-	-	11.35	2	
iodofenphos 10.0%	0	47	0	-	-	-	82	90	91	57	79	72	53	67	79	102	102	100	-	-	-	10	-	-	65.26	3	
pirimiphos methyl 1.0%	-	-	-	-	-	-	2	46	4	146	154	95	85	85	100	143	143	100	-	-	-	20	-	-	27	3	
fenthion 2.5%	-	-	-	-	-	-	-	-	-	29	29	100	-	-	-	60	60	100	-	-	-	-	-	-	-	-	-
fenitrothion 1.0%	-	-	-	5	75	7	67	118	57	251	336	75	54	61	89	447	454	99	-	-	-	17	-	-	22.16	3	
phenothate 10.0%	-	-	-	4	68	6	59	92	64	58	77	75	83	87	95	-	-	-	-	-	-	15	-	-	17.35	2	
malathion 5.0%	-	-	-	0	34	0	12	100	12	393	424	93	423	454	93	233	233	100	-	-	-	20	-	-	69.30	3	
malaoxon 5.0%	-	-	-	-	-	-	0	6	0	11	79	14	71	103	69	42	65	65	142	150	95	44	-	-	19.72	2	
propoxur 0.1%	-	-	-	-	-	-	28	75	37	120	128	-	25	31	81	341	341	100	-	-	-	16	-	-	91.26	2	
permethrin 0.2%	-	-	-	-	-	-	18	23	78	258	286	90	76	132	58	230	238	97	-	-	-	0.4	-	-	17.7	2	
decamethrin 0.001%	-	-	-	-	-	-	-	-	-	10	17	59	33	62	53	167	206	81	17	17	100	36	-	-	6.93	2	
DDT 4.0%	-	-	-	-	-	-	54	54	100	79	79	100	-	-	-	126	126	100	-	-	-	-	-	-	-	-	

D = number dead T = total exposed % = percentage mortality

Table 30. Results of laboratory exposures of *Anopheles albimanus* (F52INS/101)

from El Salvador to various insecticides for varying times

Insecticides	Exposure time in minutes																		LT <sub>50</sub> min	χ <sup>2</sup>	DF									
	3			7.5			15			30			45			60						120			180			240		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%
chlorphoxim 4.0%	-	-	-	74	119	62	11	54	20	11	46	24	126	211	60	-	-	-	-	-	-	-	-	-	120	46.01	2			
phoxim 2.5%	-	-	-	-	-	-	61	131	47	64	96	67	74	83	89	-	-	-	-	-	-	-	-	-	32	2.12	1			
iodofenphos 10.0%	-	-	-	-	-	-	-	-	-	-	-	-	43	158	27	10	53	19	-	-	-	63	78	81	131	21.27	1			
pirimiphos methyl 1.0%	-	-	-	21	64	33	2	31	7	48	95	51	185	319	54	80	105	76	-	-	-	-	-	-	45	17.51	3			
fenthion 2.5%	-	-	-	16	31	52	38	38	100	-	-	-	118	118	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
fenitrothion 1.0%	-	-	-	-	-	-	-	-	-	-	-	-	21	155	14	20	75	27	-	-	-	51	90	57	209	0.75	1			
phenothiaz 10.0%	-	-	-	-	-	-	-	-	-	18	112	16	18	74	24	38	68	56	-	-	-	23	51	45	179	10.91	2			
malathion 5.0%	-	-	-	-	-	-	47	355	13	0	16	0	454	1164	39	326	740	44	18	31	58	220	276	80	109	59.06	4			
malaoxon 5.0%	-	-	-	1	19	5	34	223	15	-	-	-	109	456	24	8	71	11	22	94	23	10	39	26	-	11.81	4			
proposur 0.1%	-	-	-	-	-	-	-	-	-	-	-	-	28	61	46	43	95	45	11	29	38	11	28	39	> 240	-	-			
permethrin 0.2%	-	-	-	107	155	69	298	343	87	137	156	88	246	266	93	21	22	96	-	-	-	-	-	-	6	2.97	3			
decmethrin 0.101%	-	-	-	23	57	40	101	133	76	51	71	72	286	336	85	89	09	100	-	-	-	-	-	-	18	11.59	3			
DUR 4.0%	1	19	1	65	212	31	169	188	90	21	64	33	307	333	92	-	-	-	-	-	-	-	-	-	20	160.76	3			

D = number dead; T = total exposed; % = percentage mortality



Fig.24

Resistance spectra of two populations of *A.albimanus* from Panama (PALB) and El Salvador (FERN5/RR).

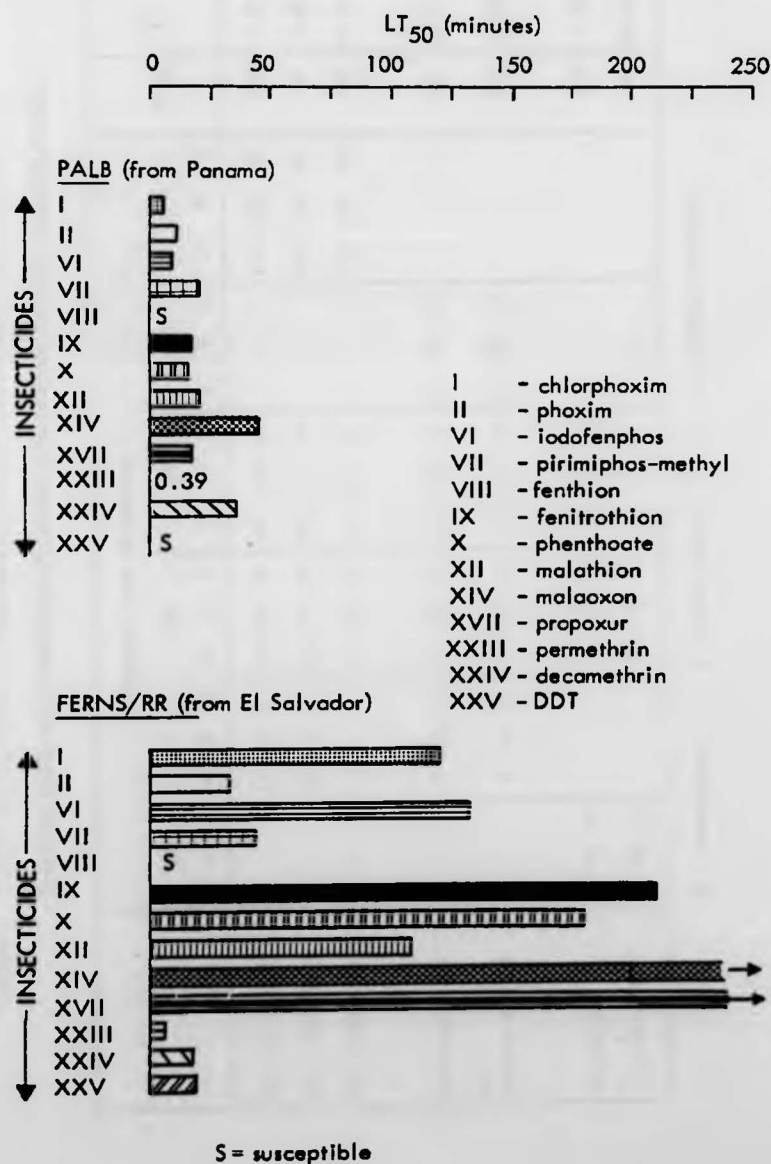


Table 11. Results of exposures of the FEHNS/RR strain of A. albimanus from El Salvador, to malathion alone and to malathion following pretreatments with TPP, PB, DEF and SV

	Exposure time in minutes															LT <sub>50</sub>	χ <sup>2</sup>	DF	SR			
	30			45			60			120			180							240		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%					D	T	%
malathion 5.0%	-			11	66	17	73	232	32	186	392	47	-			116	147	79	116	5.92	2	-
TPP + malathion	-			18	64	28	84	211	40	238	351	68	-			128	154	83	78	1.49	2	1.49
PB + malathion 5.0%	-			-			33	130	25	145	233	62	-			94	139	68	106	12.64	1	1.09
malathion 5.0%	-			5	34	15	42	157	27	81	264	31	74	116	64	-			172	18.12	2	-
DEF + malathion 5.0%	-			9	39	23	33	145	23	77	231	33	98	154	64	-			154	13.20	0	1.12
malathion	3	17	18	-			5	31	16	24	68	35	-			-			267	1.02	1	-
SV <sub>1</sub> + malathion	3	16	19	-			19	43	44	35	59	59	-			-			66	10.06	1	4.05

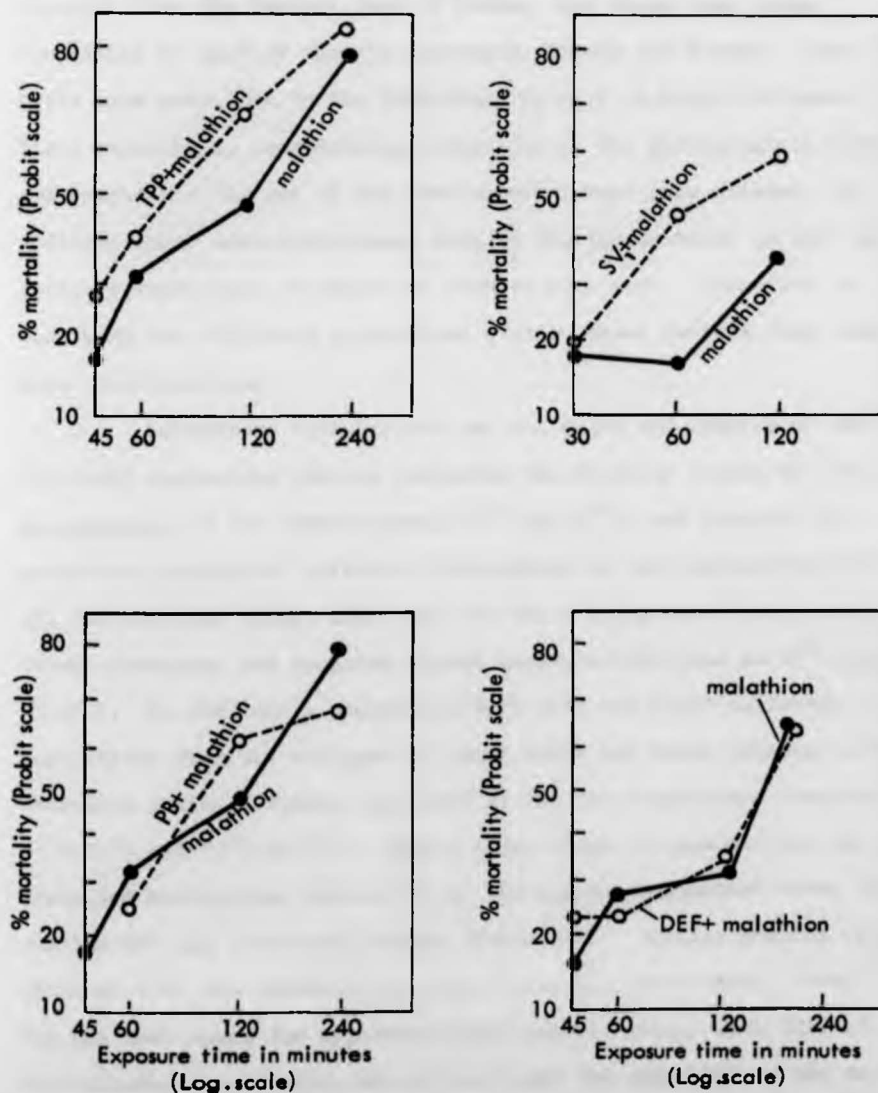
D = number dead

T = total exposed

% = percentage mortality

Fig.25

The mortality relationship in *A.albimanus* (FERNs/RR) exposed to malathion alone and to malathion after previous exposure to the synergists TPP, SV, PB and DEF.



A. sacharovi:

A number of populations of this species were compared for their response to a series of insecticides involving the OPs, carbamates, organochlorines, and the pyrethroids. Among the populations were the laboratory maintained and presumably susceptible strain Soysallı from the Central part of Turkey, and those from three localities in each of the two countries, Greece and Turkey. Since the tests were made both in the laboratory as well as under different field conditions, considerable variations in the physiological state and presumably the age of the test material must have existed. In addition there were differences both in the temperatures as well as relative humidities at which the studies were made. Therefore, in comparing the different populations studied these factors were taken into consideration.

Laboratory tests on one day old males and females of seven different anopheline species including the Soysallı strain of (Table 32) A. sacharovi at two temperatures, 20°C and 27°C, had revealed the positively correlated effect of temperature on test mortalities with all insecticides except DDT. All the tests using malathion, fenitrothion, fenthion, and propoxur showed higher mortalities at 27°C than at 20°C. In addition a comparable test with two field collected populations from the villages of Asagi kulak and Kukuk karatas in the Chukurova plain of Turkey were made at the two temperature ranges of 21°C-27°C and 31°C-33.5°C. Here a wider range of insecticides was used. Increased mortalities were shown at the higher temperature range with all the OPs and carbamates tested (Table 33). Similar results were obtained with the pyrethroids, permethrin, and decamethrin, except for one test where the opposite effect was observed. With DDT, in one village, Asagi kulak, mortalities were the same (2%) at the two

Table 32. Results of laboratory exposures of the Soysalif strain of *A. gacharovi* to different insecticides at the two temperatures 20°C and 27°C

Insecticide	Percentage mortalities (numbers tested) at one hour exposure.	
	temperature = 20°C	temperature = 27°C
fenthion	67 (33)	100 (182)
fenitrothion	10 (46)	74 (159)
malathion	85 (187)	100 (125)
propoxur	45 (197)	64 (418)

temperature ranges, while in Kukuk karatas, mortality increased from 4.0% to 10% with 4% DDT with the increase in the temperature. In this instance, however, the level of resistance was so high that further studies may be necessary to establish the effect of temperature on mortality with this insecticide. Earlier studies by other workers on the effect of temperature on DDT toxicity have usually reported a negatively correlated effect of this insecticide.

Table 33 and fig. 26 compare the response towards some insecticides shown by the populations from the three villages Sufli, Poros and Anthili of Greece with that of two localities in the Chukurova plain of Turkey. These represent the closest approximation in the test temperature ranges ( $19^{\circ}$ - $25^{\circ}$ C and  $21^{\circ}$ - $27^{\circ}$ C) that is available for comparison in the field collected populations of this species. It is evident from the results, that the populations from Greece are more susceptible towards all insecticides tested than those from Turkey, in spite of the lower test temperatures in the former country. The A. sacharovi populations from Greece can in fact be considered susceptible towards all the organophosphates tested. With the respective discriminating dosages of the insecticides the mortalities varied from 94-100% (Tables 34 to 36). The high susceptibility was most evident with malathion where in two of the localities a 45 and a 30 minute exposure was sufficient to produce a 100% mortality on the 5% concentration of the insecticide. With fenitrothion however a higher proportion of survivors were encountered, with mortalities ranging from 25-75% on 1.0% concentration for one hour. The highest mortality was from Sufli. Extending the period of exposure to two hours resulted in the population from Poros village showing an

**Table 11.** Results of field exposures of *A. sacharovi* from different localities in Greece and Turkey at varying temperature and humidity ranges (% mortality at one hour exposure) (numbers exposed are given in parentheses)

Insecticides	Temperature = 19°-25°C Relative humidity = 52%-78%			Temperature = 21°C-27°C Relative humidity = 44%-51%		Temperature = 31°C-33.5°C Relative humidity = 50%-60%	
	Sufli	Poros	Anthili	Asagi Kulak	Kucuk Karatas	Asagi Kulak	Kucuk Karatas
chlorphoxim	94 (33)	-	100 (90)	72 (50)	54 (24)	100 (76)	82 (27)
phoxim	-	-	-	67 (24)	43 (23)	100 (24)	76 (25)
mecabam	-	-	-	26 (19)	-	91 (76)	-
iodofenphos	-	-	-	38 (120)	8 (26)	80 (56)	46 (24)
pirimiphos-methyl	96 (28)	-	100 (113)	85 (27)	85 (46)	98 (120)	84 (25)
fenthion	100 (25)	100 (13)	97 (107)	-	64 (22)	-	100 (22)
fenitrothion	75 (24)	31 (127)	25 (104)	2 (213)	2 (46)	33 (225)	50 (48)
malathion	100 (27)	100 (39)	100 (88)	90 (170)	90 (60)	100 (193)	96 (46)
propoxur	100 (29)	90 (77)	86 (103)	59 (130)	59 (29)	87 (68)	88 (48)
carbaryl	-	-	-	-	35 (23)	-	27 (33)
dimetilan	-	-	-	-	11 (27)	-	31 (26)
3-isopropyl phenyl N-methyl carbamate	-	-	-	-	19 (26)	-	67 (27)
permethrin	-	-	-	61 (41)	62 (21)	92 (73)	85 (26)
decamethrin	-	-	-	0 (14)	11 (28)	26 (23)	0 (27)
DDT	97 (35)	31 (42)	61 (99)	2 (173)	4 (52)	2 (83)	10 (49)
dieldrin	75 (24)	41 (46)	41 (116)	-	-	-	0

Fig. 26

Resistance spectra of field populations of *A. sacharovi* from Greece and Turkey.

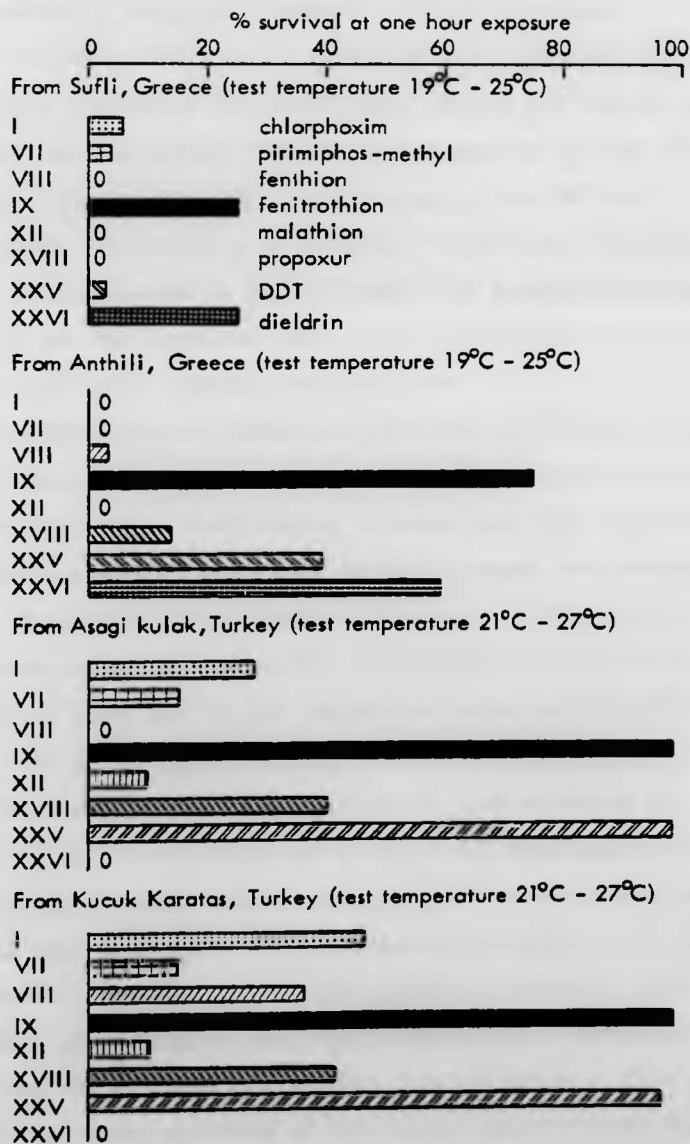
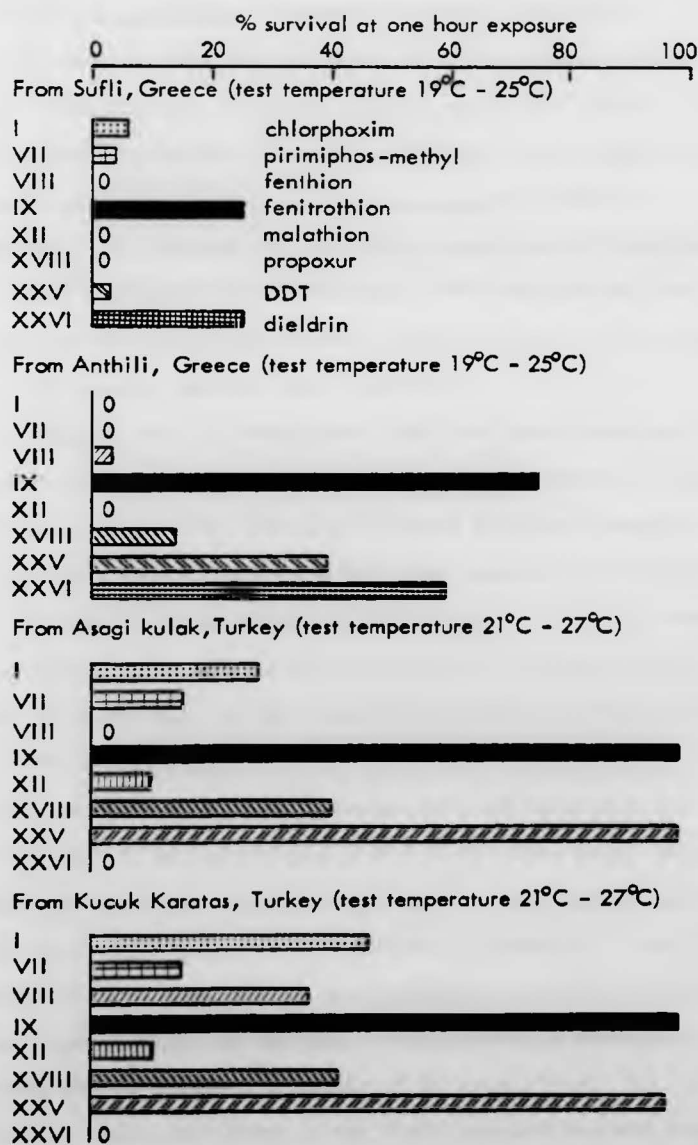




Fig.26

Resistance spectra of field populations of *A.sacharovi* from Greece and Turkey.



increase in mortality from 31% to 91% (Table 35) Therefore it may be possible that if the population from Sufli, where one hour exposure produced a 75% mortality had been exposed for two hours a higher mortality would have resulted. Laboratory studies on the discriminating dosages of different insecticides, have reported that in some species including A. sacharovi (Soysallı strain) a two hour exposure to 1% fenitrothion may be necessary for a complete kill of the susceptible population (Technical Report Series 585 W.H.O., 1976). On this basis the populations from these villages can to some extent be considered highly susceptible to fenitrothion. With DDT and dieldrin however, the presence of a certain proportion of resistant individuals were indicated in all villages. The populations from the village Sufli was the least resistant. The proportion of resistant individuals also varied between the localities.

It appears that in comparison with the above susceptibilities in the Greek populations, the lower mortalities encountered in the tests on the populations from Turkish villages (all test temperatures being higher) may suggest resistance in these towards the insecticides concerned. This may be most apparent with regard to the survivors of the discriminating dosages of OP's, carbamates, and pyrethroids particularly on tests made at the temperature range of 31°C-33.5°C (Tables 37 - 39 & Fig. 28).

The few survivors from exposures to 5.0% malathion for one hour (1 out of 291, 2 out of 191 and 2 out of 365 from Asagi kulak, Kukuk karatas and Tabaklar respectively) were further investigated to establish if they represented true resistant individuals. The progeny from the eggs obtained from one of the malathion survivors, further selected for 4 generations at the same dosage showed a reduction in mortality from 99% to 85.7% on a sample of 24 mosquitoes. This seems to indicate that those survivors in the field populations may represent

the resistant individuals. However, further selections from more such survivors would be necessary to confirm this. In addition, the 90% mortality (10% survival) that was encountered in two of the villages from Turkey (Table 33) on field exposures, particularly when compared to the 100% kill even at 30 and 45 minute exposures at a still lower test temperature ( $19^{\circ}\text{C}$ - $25^{\circ}\text{C}$ ) in the Greek populations may also be of some significance. This may need careful consideration in view of the additional and direct selection pressure now exerted on this population from the use of this insecticide in the Malaria Control Programme.

The survivors from fenthion and propoxur discriminating dosages were similarly demonstrated as representing resistant individuals, through subsequent selection of the progenies with the relevant insecticides. With fenthion an initial mortality of 87% was reduced to 58%, while with propoxur a change in mortality from 88% to 36% occurred. Surprisingly, propoxur resistance was also evident in the supposedly susceptible Soysalli strain where selection with 0.1% propoxur at one hour reduced the mortality.

The resistance towards fenitrothion was more obvious. An exposure of 4 hours to the 1.0% concentration gave mortalities ranging from 67-84% in spite of the high test temperatures (Tables 37-39). Similar resistance levels were also shown towards DDT, dieldrin and carbaryl.

High tolerances, when compared with most of the other anopheline species tested towards the two pyrethroids, permethrin and decamethrin, were recorded in all three villages in spite of the fact that these compounds had never been used in the field. Survivors were encountered at two hour exposures to 0.2% permethrin even in tests carried out at the higher temperature ranges. The mortalities at one

Table 14. Results of field exposures of *Anopheles sacharovi* from Safli, Nomos Evros in Greece to various insecticides for varying times

insecticides	Exposure time in minutes												LT <sub>50</sub> in minutes	χ <sup>2</sup>	DF			
	7.5			15			30			60						120		
	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%
chlorphoxim 4.0%		-		1	23	0	8	11	73	31	33	94		-		28	1.91	1
pirimiphos methyl 1.0%		-		3	14	21	3	13	100	27	28	96		-		29	7.08	1
fenthion 2.5%		-		11	16	69	7	7	100	25	25	100		-		-	-	-
fenitrothion 1.0%	0	17	0	1	16	0	14	19	74	18	24	75		-		30	7.38	2
malathion 5.0%	0	5	0	10	14	71	26	26	100	27	27	100		-		-	-	-
propoxur 0.1%		-		3	7	43	0	3	0	29	29	100		-		-	-	-
DDT 4.0%		-		-			6	12	50	34	35	97	1	2	50	23	37.65	1
dieldrin 4.0%		-		-			66	118	56	18	24	75	18	18	100	27	1.75	1

D = number dead    T = total exposed    % = percentage mortality

Table 15. Results of field exposures of *Anopheles sacharovi* from Poros.

Mumukh, Evros in Greece, to various insecticides for varying times

Insecticides	Exposure time in minutes															LT <sub>50</sub> in mins	χ <sup>2</sup>	DF												
	0.3			7.5			15			30			45						60			120			240			960		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%	D	T	%	D	T	%	D	T	%
fenthion 2.5%	0	34	0	3	34	9	32	39	82	39	43	91	-	-	-	13	13	100	-	-	-	-	-	-	-	-	12	9.89	3	
fenitrothion 1.0%	-	-	-	-	-	-	-	-	-	6	50	12	39	127	31	133	146	91	-	-	-	-	-	-	-	-	72	0.11	1	
malathion 5.0%	-	-	-	-	-	-	30	47	64	102	112	91	66	66	100	139	139	100	-	-	-	-	-	-	-	-	13	2.56	2	
propoxur 0.1%	-	-	-	-	-	-	8	31	26	11	18	61	-	-	-	69	77	90	-	-	-	-	-	-	-	-	24	0.0	1	
DDT 4.0%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	42	31	11	36	31	41	77	53	81	91	89	183	3.60	2
dieldrin 4.0%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19	46	41	32	51	63	55	78	71	55	72	76	63	2.96	2

D = number dead

T = total exposed

% = percentage mortality

Table 36. Results of field exposures of *Anopheles sacharovi* from Anthili, Lemna Plain of Greece to various insecticides for varying times

Insecticides	Exposure time in minutes																					LT <sub>50</sub> in mins	$\chi^2$	DF
	3			7.5			15			30			60			120			240					
	D	T	%	S	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%			
chlorphoxim 4.0%	5	21	24	43	58	74	93	110	85	70	83	84	90	90	100	-	-	-	-	-	-	4	14.58	3
pirimiphos methyl 1.0%	9	52	17	14	58	24	63	107	59	80	91	88	113	113	100	-	-	-	-	-	-	11	14.47	3
fenthion 2.5%	-	-	-	-	-	-	36	107	34	44	70	63	104	107	97	-	-	-	-	-	-	21	4.14	1
fenitrothion 1.0%	-	-	-	-	-	-	-	-	-	5	86	6	26	104	25	64	101	63	93	100	93	93	0.49	2
malathion 5.0%	9	40	23	64	91	70	89	97	92	88	88	100	88	88	100	-	-	-	-	-	-	5	0.99	3
propoxur 0.1%	0	26	0	-	-	-	7	41	17	53	125	42	89	103	86	-	-	-	-	-	-	32	2.26	2
DDT 4.0%	-	-	-	-	-	-	-	-	-	36	84	43	60	99	61	56	91	62	91	98	93	44	11.66	2
dieldrin 4.0%	-	-	-	-	-	-	-	-	-	-	-	-	48	116	41	40	93	43	58	92	63	125	2.19	1

D = number dead    T = total exposed    % = percentage mortality

Table 37. Results of field exposures of *Anopheles sacharovi* from Asagi Kolak, Chukurova Plain of Turkey to various insecticides for varying times

Insecticides	Exposure time in minutes															LT <sub>50</sub> ln mins	χ <sup>2</sup>	DF			
	15			30			60			120			240						360		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%
chlorphoxin 4.0%	116	142	82	102	110	93	193	193	100	-	-	-	-	-	-	7	3.12	1			
phoxin 2.5%	10	25	40	150	175	86	100	101	99	26	26	100	-	-	-	17	0.02	2			
parathion 1.0%	16	23	70	63	81	78	141	164	86	27	27	100	-	-	-	8	2.41	2			
diazinon 0.1%	3	25	12	13	24	54	81	90	90	20	25	80	19	19	100	28	22.44	3			
mecabam 10.0%	-	-	-	16	24	67	69	76	91	-	-	-	-	-	-	-	-	-			
iodofenphos 10.0%	55	91	60	85	154	55	116	136	85	46	49	94	-	-	-	15	15.82	2			
pirimiphos methyl 1.0%	100	134	75	62	63	98	118	120	98	-	-	-	-	-	-	8	3.44	1			
fenthion 2.5%	33	70	47	89	131	68	118	120	98	25	25	100	-	-	-	18	8.78	2			
fenitrothion 1.0%	-	-	-	30	148	20	74	225	33	102	133	77	46	69	67	86	24.75	2			
malathion 5.0%	229	305	75	256	259	99	290	291	100	-	-	-	-	-	-	10	7.81	1			
bimyl 1.0%	32	46	70	21	25	84	47	47	100	-	-	-	-	-	-	11	2.13	1			
dimethoate 1.0%	-	-	-	43	70	61	81	94	86	-	-	25	25	100	-	2	0.09	1			
EFN 1.0%	15	19	79	75	79	95	63	68	93	200	211	95	-	-	-	1	3.22	2			
propanox 0.1%	86	133	65	112	158	71	192	213	90	50	51	98	-	-	-	10	5.57	2			
carbaryl 5.0%	-	-	-	13	62	21	71	164	43	48	92	52	38	44	86	83	5.11	2			
pyrolex 5.0%	17	24	71	20	46	43	114	115	99	-	-	-	-	-	-	18	37.92	1			
dimeton 2.0%	44	54	81	47	51	92	76	76	100	-	-	-	-	-	-	7	1.47	1			
dimetilan 2.0%	8	52	15	63	92	68	52	52	100	-	-	-	-	-	-	24	1.15	1			
5-isopropyl phenyl N-methyl carbamate 0.001%	1	23	4	35	47	74	74	81	91	45	47	96	-	-	-	25	13.53	2			
permethrin 0.2%	106	169	63	94	109	86	126	138	91	50	51	98	23	23	100	10	2.15	3			
decamethrin 0.001%	1	23	4	3	51	6	13	74	18	42	102	41	-	-	13	26	50	219	4.81	3	
DDT 4.0%	-	-	-	0	44	0	15	211	7	21	219	10	25	44	57	-	308	16.89	2		
dieldrin 4.0%	-	-	-	1	24	4	27	106	25	27	63	43	7	24	29	-	289	7.32	2		

D = number dead T = total exposed % = percentage mortality

Table 28. Results of field exposures of *Anopheles sacharovi* from Enlik Karatan, Çankırı Province of Turkey to various insecticides for varying times

Insecticides	Exposure time in minutes															LT <sub>50</sub> in minutes	χ <sup>2</sup>	DF
	15			30			60			120			240					
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%			
chlorphoxim 4.0%	49	78	63	54	64	84	103	111	93	28	28	100	-	-	-	10	0.82	2
phoxim 2.5%	19	44	43	51	65	79	100	100	93	49	98	50	-	-	-	-	-	-
parathion 1.0%	-	-	-	3	22	14	33	35	94	-	-	-	-	-	-	-	-	-
diazinon 0.1%	0	22	0	11	50	22	19	68	28	16	21	76	16	18	89	82	6.83	3
mecapton 10.0%	13	28	46	17	19	90	22	22	100	-	-	-	-	-	-	16	0.10	1
iodofenphos 10.0%	11	44	25	20	78	36	32	56	57	76	80	95	25	25	100	30	10.56	3
pirimiphos methyl 1.0%	44	81	54	140	148	95	117	121	97	51	51	100	-	-	-	13	10.16	2
fenitrothion 2.5%	22	75	29	28	42	69	58	80	73	49	49	100	28	28	100	25	0.99	3
fenitrothion 1.0%	-	-	-	1	20	35	43	131	33	56	99	57	33	46	72	101	3.18	2
malathion 5.0%	75	85	88	152	158	96	109	191	99	-	-	-	-	-	-	3	0.01	1
bomyl 1.0%	18	44	41	26	26	100	24	24	100	-	-	-	-	-	-	17	5.35	2
dimethoate 1.0%	0	25	0	2	44	5	16	59	27	6	24	25	-	-	-	-	-	-
DFP 1.0%	-	-	-	16	25	64	43	49	88	20	21	95	-	-	-	20	0.18	1
propoxur 0.1%	38	66	58	45	59	76	54	60	90	25	26	96	-	-	-	12	0.02	2
carbaryl 5.0%	-	-	-	6	20	30	73	169	43	12	20	45	-	-	-	165	0.72	1
pyrethrin 5.0%	-	-	-	12	15	80	-	-	-	32	32	100	-	-	-	-	-	-
dimeton 2.0%	4	27	15	-	-	-	85	112	76	29	29	100	-	-	-	35	1.69	1
dimethilan 2.0%	17	26	65	22	27	82	36	54	67	20	23	87	64	64	100	11	14.20	3
3-isopropyl phenyl N-methyl carbamate 0.001%	1	30	3	25	42	60	88	102	86	25	28	89	-	-	-	32	17.13	2
permethrin 0.2%	12	22	55	75	109	69	70	83	84	51	54	94	-	-	-	15	0.25	2
decamethrin 0.001%	-	-	-	-	-	-	3	56	5	12	43	20	31	75	41	208	1.69	1
DUP 4.0%	-	-	-	0	4	0	9	82	11	21	56	38	33	44	75	149	0.09	2
Bieldrin 4.0%	-	-	-	-	-	-	13	29	45	29	52	56	-	-	-	-	-	-

D = number dead      T = total exposed      % = percentage mortality



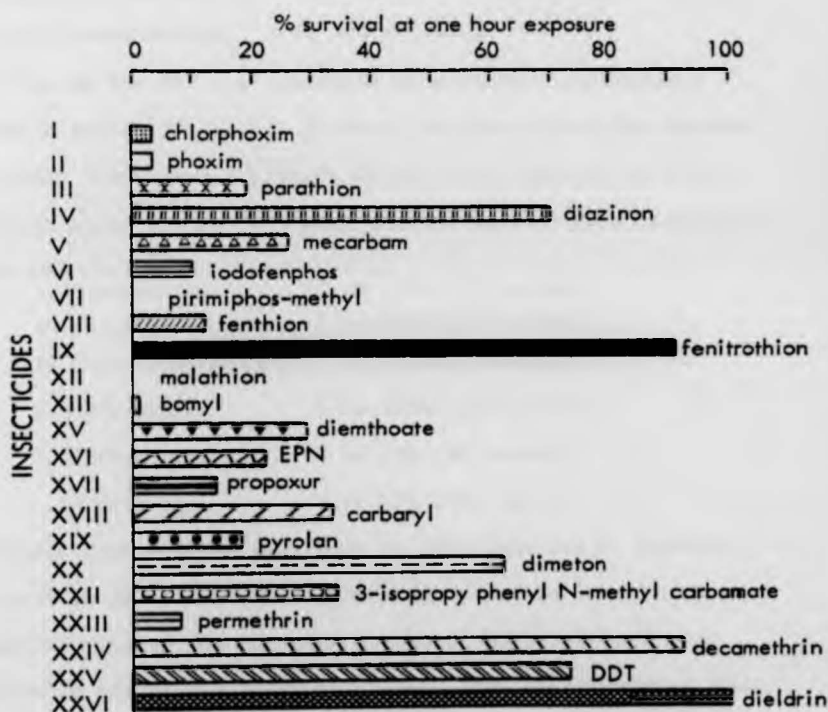
Table 39. Results of field exposures of *Anopheles sacharovi* from Tabaklar, Chakurova Plain of Turkey to various insecticides for varying times

Insecticides	Exposure time in minutes															LT <sub>50</sub> in mins	$\chi^2$	DF			
	15			30			60			120			240						360		
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%
chlorphoxin 4.0%	77	102	75	77	90	86	173	178	97	-	-	-	-	-	-	7	1.46	1			
phoxin 2.5%	42	57	74	67	81	85	74	76	97	25	25	100	-	-	-	8	2.43	2			
parathion 1.0%	2	26	8	56	80	70	43	53	81	35	41	85	-	-	-	24	15.90	2			
diazinon 0.1%	0	22	0	0	23	0	13	43	50	-	-	-	-	-	-	80	0.04	1			
secabur 10.0%	0	25	0	1	50	2	55	74	74	-	-	-	-	-	-	-	-	-			
iodofenphos 10.0%	31	84	57	20	57	35	142	158	90	25	25	100	-	-	-	24	20.64	2			
pirimiphos methyl 1.0%	56	114	49	45	68	66	153	153	100	-	-	-	-	-	-	17	16.01	1			
fenthion 2.5%	19	57	53	56	111	50	128	145	88	75	77	97	-	-	-	25	5.27	2			
fenitrothion 1.0%	-	-	-	2	30	7	4	47	9	58	115	50	64	76	84	-	121	4.63	2		
malathion 5.0%	99	110	90	124	128	97	363	365	100	-	-	-	-	-	-	4	0.04	1			
bosyl 1.0%	17	28	61	11	18	61	74	75	99	29	29	100	-	-	-	14	7.90	2			
disethoate 1.0%	11	77	14	2	26	8	61	86	71	39	78	90	-	-	-	67	33.28	2			
KPH 1.0%	-	-	-	-	-	-	31	40	78	27	27	100	-	-	-	-	-	-			
propoxur 0.1%	28	64	44	20	48	42	168	195	86	49	56	88	-	-	-	21	14.53	2			
carbaryl 5.0%	3	48	6	30	56	68	18	27	67	25	25	100	-	-	-	30	18.02	2			
pyrolan 5.0%	-	-	-	-	-	-	22	27	82	27	27	100	-	-	-	-	-	-			
dimetan 2.0%	-	-	-	2	20	10	8	21	58	26	27	96	-	-	-	61	1.93	1			
5-isopropyl phenyl N-methyl carbamate 0.001%	5	25	20	13	25	52	36	55	66	31	31	100	-	-	-	34	5.44	2			
permethrin 0.2%	13	25	52	82	98	84	125	136	92	50	53	94	43	43	100	-	10	3.87	3		
decamethrin 0.001%	-	-	-	-	-	-	4	53	8	5	49	10	9	23	39	31	55	56	323	2.35	2
DDE 4.0%	-	-	-	0	50	0	14	52	27	17	96	18	47	84	56	-	232	19.14	2		
dieldrin 4.0%	-	-	-	-	-	-	0	21	0	19	83	23	18	56	50	13	30	43	314	4.86	2

D = number dead      T = total exposed      % = percentage mortality

Fig.28

Resistance spectrum of a field population of  
*A.sacharovi* from Tabaklar, Chukurova Plain,  
Turkey.



hour exposure in the tests done at 21°C-27°C were 61% and 62% in material from the villages of Asagi kulak and Kukuk karatas. With decamethrin, a similar high level of resistance was observed. Zero and 11% mortalities were shown at one hour with the 0.001% concentration when tested at 21°C-27°C. Even in tests made at high temperatures, the percentage mortality was not increased beyond 50-56% in exposures for 6 hours to this concentration. In the Soysalli

strain as far as the OP's are concerned, chlorphoxim, pirimiphos-methyl, fenthion and malathion all produced complete mortality whereas with fenitrothion there were survivors at one hour exposure to 1.0% concentration as shown by the following figures derived from laboratory

tests carried out at 27°C:-	
5.0% malathion	1 hr 100% (55 tested)
4% chlorphoxim	1 hr 100% (44 tested)
1% pirimiphos methyl	1 hr 100% (9 tested)
2.5% fenthion	1 hr 100% (120 tested)
1.0% fenitrothion	1 hr 67% (20 tested)
0.1% propoxur	1 hr 72% (156 tested)

Limited observations were made on small samples of material using synergists in an attempt to identify the possible detoxication mechanism contributing to the resistance towards DDT, fenitrothion, and iodofenphos in the A. sacharovi population from the Chukurova plain of Turkey. F-DMC, PB, and sesamex all synergised DDT but to a limited extent, in tests made both in the laboratory as well as in the field collected samples. In the laboratory tests a mortality of 3.0% at a one hour exposure to 4.0% DDT, was increased by pretreatment with F-DMC to 24%. Similarly PB and sesamex enhanced the toxicity of DDT again from 3.0% mortality to 17% and 18% respectively. With the two hour exposures however, there was no further increase in the toxicity of DDT, when combined with either F-DMC or PB. Similarly as shown in Table 40 in the field observations the one hour exposures increased

the 24% mortality of DDT alone, to 45% and 41% on pretreatments with PB and F-DMC respectively. Exposures at two hours again showed synergism, the 24% mortality produced by DDT alone being increased by PB and F-DMC to 52% and 72% respectively. On the other hand at the 4 hour exposure, there was no effect of F-DMC on enhancing the toxicity of DDT. PB was not used at this exposure time.

Synergism to some extent, shown by F-DMC, PB, and sesamex may all suggest that at least two detoxication mechanisms, a DDT-ase and one involving the mfo system, may be contributing to the DDT resistance in this population. However it is clearly evident from both observations, that none of the synergists were effective beyond a certain level and therefore the resistance could not be overcome to any great extent by the synergists.

The quantity of the synergists being insufficient to cope with the high detoxication capacity in these populations can be considered as one of the possible explanations for this. On the other hand if the comparable tests made on other species such as A. culicifacies CUL/SRL, (Table 5, fig. 3) or A. stephensi ST/BAR (Table 23 and fig. 19) are considered, then it is possible to presume that additional factors may be involved in the DDT resistance in this A. sacharovi population, which is not being affected by either of the two types of the synergists concerned. Although the observations made are both incomplete and insufficient, in view of the resistance (possibly cross resistance) shown by this population towards the pyrethroids, permethrin and decamethrin it is tempting to suggest that the mechanism could be of the nature of a knockdown resistance factor.

Table 40. Results of laboratory and field exposures of *A. sacharovi* from Chukurova Plain of Turkey to DDT alone and to DDT following pre-treatments with PB, F-DMC, and sesamex

compounds and concentration	Exposure time in minutes								
	60			120			240		
	D	T	%	D	T	%	D	T	%
<u>laboratory tests:</u>									
DDT 4.0%	22	795	3	24	44	55	-		
PB + DDT 4.0%	49	283	17	49	94	52	-		
F-DMC + DDT 4.0%	67	285	24	30	51	59	-		
sesamex + DDT 4.0%	29	163	18	-			-		
<u>field tests:</u>									
DDT 4.0%	12	50	24	14	67	21	14	19	74
PB + DDT 4.0%	32	71	45	49	94	52	-		
F-DMC + DDT 4.0%	42	102	41	54	75	72	13	23	57

D = number dead, T = total exposed, % = percentage mortality

Table 41 \* Results of field and laboratory exposures of *A. sacharovi*  
from Chukurova Plain of Turkey to fenitrothion alone and  
to fenitrothion following pretreatments with PB, sesamex,  
S421, DEF and SV<sub>1</sub>

Compounds and concentrations	Exposure time in minutes					
	60			120		
	D	T	%	D	T	%
<u>Field tests</u>						
fenitrothion 1.0%	21	251	5	3	21	14
PB + fenitrothion 1.0%		-		10	23	43
sesamex + fenitrothion 1.0%	26	52	50	19	27	70
S421 + fenitrothion 1.0%	53	136	39	19	22	86
DEF + fenitrothion 1.0%	57	160	36	20	25	80
SV <sub>1</sub> + fenitrothion 1.0%	18	50	36		-	
<u>Laboratory tests</u>						
fenitrothion 1.0%	5	394	1		-	
PB + fenitrothion 1.0%	4	29	14		-	
sesamex + fenitrothion 1.0%	21	28	75		-	
S421 + fenitrothion 1.0%	21	84	25		-	
DEF + fenitrothion 1.0%	10	85	12		-	

D = number dead    T = total exposed    % = percentage mortality

Table 42. Results of field exposures of *A. sacharovi* from Chukurova Plains of Turkey to iodofenphos alone and to iodofenphos following pretreatments with S421, DEF and SV<sub>1</sub>

Compounds and concentration	Exposure time = 60 minutes		
	D	T	%
iodofenphos 10.0%	46	113	41
S421 + iodofenphos 10%	23	51	45
DEF + iodofenphos 10%	59	78	76
SV <sub>1</sub> + iodofenphos 10%	22	30	73

D = number dead, T = total exposed, % = percentage mortality

With fenitrothion resistance, both laboratory and field observations have shown synergism with all the synergists used, viz. PB, sesamex, S421, SV<sub>1</sub> and DEF (Table 41). This evidence therefore suggest the possible involvement of at least the mixed function oxidase system as well as the hydrolytic esterases in this resistance.

With iodofenphos only a single dose i.e. one hour exposure to 10.0% concentration was used with the synergists. Here S421 (Table 42) showed no synergism, while SV<sub>1</sub> and DEF both produced increased toxicity of the insecticide. Here the effect of the latter may suggest the possible involvement of the hydrolytic esterases in this resistance. The synergism by SV<sub>1</sub> could indicate the importance of mfo's, but since S421 also an inhibitor of this enzyme system showed no effect further tests may be necessary for making any deductions.

It should be pointed out that the assumptions made above on the possible detoxication mechanisms concerned are made with some reservations since only limited observations were made on material which was available on one occasion and it was not possible to repeat on larger samples. Further the populations studied being multiresistant, may impose further limitations on the interpretations.

#### A. maculipennis

Populations of this species from the villages of Sufli and Poros, in Evros, Greece, were studied in the field while that from Osmanjik in Northern Turkey was tested in the laboratory. Tables 43 to 45 show the results of the tests. The conditions under which the tests were made in the field have been referred to earlier.



Table 43. Results of field exposures of *Anopheles maculipennis* from Sufli, Evros in Greece to various insecticides for varying times

Insecticide	Exposure time in minutes															LT <sub>50</sub> in minutes	χ <sup>2</sup>	DF
	7.5			15			30			60			120					
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%			
chlorphoxim 4.0%	-			15	39	39	21	30	70	47	94	50	-			32	6.27	1
pirimiphos methyl 1.0%	-			7	31	23	28	34	82	41	42	98	-			21	0.85	1
fenthion 2.5%	-			23	29	79	31	38	82	38	39	97	-			6	2.07	1
fenitrothion 1.0%	2	30	7	2	34	6	41	57	72	62	74	84	-			26	11.50	2
malathion 5.0%	2	37	5	19	38	50	67	67	100	52	52	100	-			14	2.41	2
propoxur 0.1%	-			7	34	21	18	38	47	44	49	90	-			28	1.20	1
DDT 4.0%	-			-			6	33	18	60	75	80	73	80	91	43	7.20	1
dieldrin 4.0%	-			-			24	46	52	11	14	79	47	59	80	25	0.75	1

D = number dead T = total exposed % = percentage mortality

Table 44. Results of field exposure of Anopheles maculipennis from Poros,  
Evros in Greece to various insecticides for varying times

Insecticides	Exposure time in minutes																		LT <sub>50</sub> in mins	$\chi^2$	DF													
	3			7.5			15			30			45			60						120			240			960						
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%				
fenthion 2.5%	0	5	0	0	12	0	2	6	33	4	4	100	-	-	-	2	2	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
fenitrothion 1.0%	-	-	-	-	-	-	-	-	-	-	-	-	11	38	29	32	64	50	37	37	100	-	-	-	-	-	-	58	1.82	1	-	-	-	
malathion 5.0%	-	-	-	-	-	-	37	43	86	44	44	100	10	10	100	48	48	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
propoxur 0.1%	-	-	-	-	-	-	5	11	46	1	2	50	-	-	-	9	11	82	-	-	-	-	-	-	-	-	-	18	0.19	1	-	-	-	-
DDP 4.0%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21	42	50	41	54	76	5	5	100	6	6	100	-	-	-	-	-	-	-
dieldrin 4.0%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17	31	55	23	31	74	13	14	93	15	16	94	-	-	-	-	-	-	-

D = number dead    T = total exposed    % = percentage mortality

Table 45. Results of Laboratory exposures of *A. maculipennis* from Osmanlik, Turkey, to different insecticides

Insecticide	Exposure time in minutes		
	60	120	240
chlorphoxim 4.0%	100 (7)	-	-
iodofenphos 10.0%	0 (5)	-	-
pirimiphos methyl 1.0%	100 (9)	-	-
fenthion 2.5%	100 (19)	-	-
fenitrothion 1.0%	19 (26)	89 (9)	-
malathion 5.0%	100 (30)	-	-
propoxur 0.1%	33 (30)	81 (37)	-
carbaryl 5.0%	33 (6)	-	-
permethrin 0.2%	78 (9)	90 (26)	-
decamethrin 0.001%	53 (15)	78 (18)	67 (12)

figures in parentheses represent sample size

In the populations from Greece survivors of varying proportions were encountered from exposures to all the insecticides with the exception of malathion. With fenitrothion a two hour exposure of 37 mosquitoes produced a 100% mortality. Considering the low test temperature of 19°-25°C it can be considered susceptible. With malathion a 100% mortality with the 5.0% concentration at a 30 minute exposure showed high susceptibility.

The population from Osmanjik, Turkey showed resistance towards fenitrothion, iodofenphos, propoxur, carbaryl, permethrin, decamethrin.

In general this species appears to show a resistance pattern comparable to that of A. sacharovi although at a low level.

A. superpictus:

Only one population of this species from the village Castri, in the Lamia Plain of Greece was tested (Table 46 ). Here the limited sample only allowed tests at one hour on the discriminating dosages of the respective insecticides. In comparison with most other species this population showed a high susceptibility towards almost all the insecticides, even at the low test temperature range of 19°C-25°C. A 100% mortality was obtained with 2.5% fenthion, 0.2% permethrin and 0.0025% decamethrin. With all others occasional survivors were encountered. Either these may represent the rare resistant individuals in the population or survivors at comparatively low test temperatures. In this connection the two survivors encountered in a sample of 78, after exposure to 5.0% malathion at one hour may be of significance. In A. sacharovi from the same area a 30 minute exposure with the same concentration gave a 100% mortality, tested at the same temperature range.

Table 46. Results of field exposures of *Anopheles superpictus*  
from Castri, Lamia Plain of Greece to various insecticides  
 (% of mortality for one hour)

Insecticide	Number dead	Total number	% mortality
chlorphoxim 4.0%	91	95	96
primiphos methyl 1.0%	110	111	99
fenthion 2.5%	100	100	100
malathion 5.0%	76	78	97
fenitrothion 1.0%	83	87	95
propoxur 0.1%	93	94	99
permethrin 0.2%	37	37	100
decamethrin 0.0025%	77	77	100
DDT 4.0%	91	93	98
dieldrin 4.0%	100	102	98

A. hyrcanus:

The resistance spectrum of this species from the Chukurova plain of Turkey has been studied both in the laboratory and in the field (Tables 47,48 and fig. 27 ). In the field a wider range of insecticides were tested.

The laboratory tests on one day old adults carried out at 27°C showed higher survivors than occurred in the field tests. This applied to all the insecticides. In the field the test temperatures were high (31°C-33.5°C) and the mosquitoes tested were blood fed, being collected from cattle baited net traps. Control mortalities were high in the field tests.

The field tests recorded a 100% mortality on a sample of 272 mosquitoes exposed to 5.0% malathion for one hour. A similar exposure in the laboratory however revealed a 10% survival among 112 mosquitoes tested. These survivors were confirmed as representing resistant individuals, following selection of their progenies at the same dosage. The selected sample showed only a 50.7% mortality on a sample of 140 mosquitoes compared to the 90% in the parent population.

In general, this population of A. hyrcanus appears to be resistant to almost all the OP insecticides tested. Towards the carbamates there was a high susceptibility. An exception was the carbamate dimetilan which only produced a 2% mortality. This may have been an effect of the insecticide papers concerned although this fact was not definitely established. High resistances to both DDT and dieldrin were evident.

Attempts to select the population independently with fenitrothion and fenthion did not produce any obvious increase in the resistance level to these insecticides or to propoxur.

It is presumed that this may be a result of the slow selection procedure enforced by the necessity for hand mating of the

survivors. However, it was observed that the 90.2% mortality resulting from exposures to 5.0% malathion of the unselected population was reduced to 77.2% and 85.0% mortalities in the fenitrothion and fenthion selected lines respectively. This may be of significance and therefore needs further investigation.

Two strains of the closely related species A. lesteri from the Philippines, and A. hyrcanus from Afghanistan, were tested on malathion, fenitrothion, propoxur and fenthion. With the A. hyrcanus a 100% mortality was obtained with 1.0% fenitrothion after an exposure of only 30 minutes with a sample of 10 mosquitoes. Similarly 2.5% fenthion at 30 minutes gave a 100% kill of 16 mosquitoes. A. lesteri gave a complete kill with 1.0% fenitrothion, 2.5% fenthion, 0.1% propoxur all at one hour exposures on samples of 65, 74, and 15 respectively. These susceptibilities when compared with those of the population from Chukurova Plain discussed above further confirm the resistance in that country (Turkey) towards these insecticides.

Table 47. Results of laboratory exposures of *Anopheles byrsonus* from Chukurova

Plain of Turkey to various insecticides for varying times.

Insecticides	Exposure time in minutes												LT <sub>50</sub> in mins	χ <sup>2</sup>	DF																				
	15			30			45			60						90			120			240			360			480							
	D	T	%	D	T	%	D	T	%	D	T	%				D	T	%	D	T	%	D	T	%	D	T	%	D	T	%					
chlorphoxin 4.0%	-	6	41	39	-	-	54	87	62	-	-	-	56	67	84	27	28	96	-	-	-	-	-	-	42	0.21	2								
pirimiphos methyl 1.0%	-	-	-	-	-	-	38	87	44	-	-	-	80	65	15	-	-	-	-	-	-	-	-	-	-	-	-								
fenthion 2.5%	-	-	-	-	-	-	225	729	31	-	-	-	17	129	75	70	73	96	-	-	-	-	-	-	81	0.12	1								
fenitrothion 1.0%	-	-	-	-	-	-	14	174	8	-	-	-	4	57	7	2	42	5	9	15	60	5	19	79	510	26.04	3								
chlorthion 0.1%	-	10	15	67	9	14	64	5	10	50	-	-	-	-	-	-	-	-	-	-	-	-	-	71	0.18	1									
malathion 5.0%	8	9	89	20	27	74	38	88	17	101	112	90	14	14	100	25	25	100	-	-	-	-	-	-	8	7.34	3								
propoxur 0.1%	-	16	16	100	-	-	127	129	98	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
carbaryl 5.0%	-	-	-	-	-	-	20	20	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
decamethrin 0.001%	-	1	7	14	-	-	15	24	63	-	-	-	27	40	60	19	20	95	-	-	-	-	-	59	3.44	2									
DIF 4.0%	-	-	-	-	-	-	5	68	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
dieldrin 4.0%	-	-	-	-	-	-	3	41	7	-	-	-	0	17	0	0	20	0	-	-	-	5	22	23	400	5.64	2								
N = number dead												T = total exposed												% = percentage mortality											

D = number dead

T = total exposed

% = percentage mortality



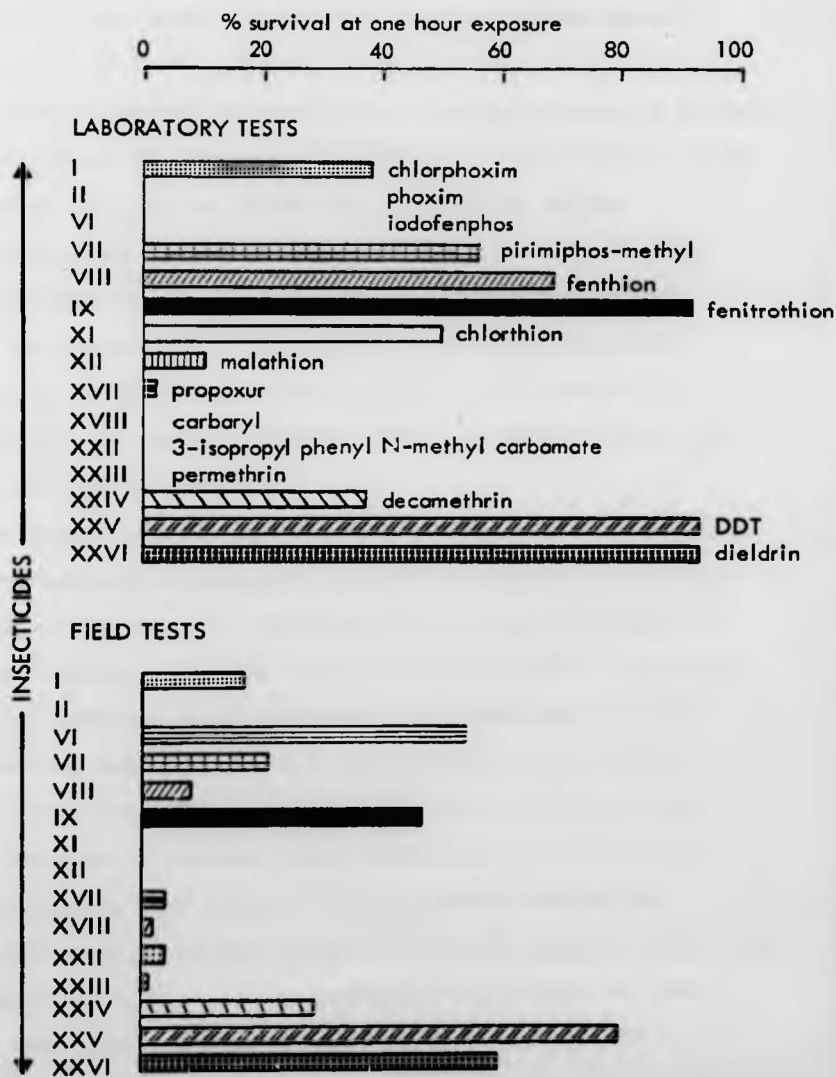
Table 48. Results of field exposures of *Anopheles hyrcanus* from Chukurova Plain of Turkey, to various insecticides for varying times

Insecticides	Exposure time in minutes															LT <sub>50</sub> in minutes	χ <sup>2</sup>	DF
	15			30			45			60			90					
	D	T	%	D	T	%	D	T	%	D	T	%	D	T	%			
chlorophen 4.0%	38	41	93	114	137	83	-	-	-	156	187	83	-	-	-	1	1.52	1
phoxin 2.5%	30	35	86	93	96	97	-	-	-	67	67	100	-	-	-	7	0.25	1
parathion 1.0%	-	-	-	-	-	-	-	-	-	31	31	100	-	-	-	-	-	-
diazinon 0.1%	-	-	-	-	-	-	-	-	-	23	44	52	-	-	-	-	-	-
iodofenphos 10%	14	47	30	52	57	91	-	-	-	88	192	46	-	-	-	-	-	-
pirimiphos methyl 1.0%	34	37	92	75	91	82	-	-	-	168	212	79	-	-	-	-	-	-
fenthion 2.5%	-	-	-	77	80	96	-	-	-	97	105	92	-	-	-	-	-	-
fenitrothion 1.0%	-	-	-	86	67	24	-	-	-	101	191	53	-	-	-	56	-	0
malathion 5.0%	143	144	99	417	419	99.5	-	-	-	272	272	100	-	-	-	1	.56	1
bomyl 1.0%	-	-	-	19	20	95	-	-	-	26	28	98	-	-	-	1	0.00	0
malaoxon 5.0%	-	-	-	-	-	-	-	-	-	5	22	23	-	-	-	-	-	-
dimethoate 1.0%	-	-	-	-	-	-	-	-	-	48	124	39	-	-	-	-	-	-
DDH	-	-	-	-	-	-	-	-	-	13	14	93	-	-	-	-	-	-
propanox 0.1%	-	-	-	68	101	67	-	-	-	127	133	96	-	-	-	23	-	-
carbaryl 5.0%	-	-	-	7	25	28	-	-	-	48	49	98	-	-	-	35	-	-
disulfilan 2.0%	-	-	-	-	-	-	-	-	-	9	39	23	-	-	-	-	-	-
5-iso propyl phenyl N-methyl carbamate 0.001%	-	-	-	1	27	4	-	-	-	102	106	96	-	-	-	42	-	-
permethrin 0.2%	-	-	-	14	23	61	-	-	-	176	177	99	-	-	-	28	-	-
decamethrin 0.001%	-	-	-	-	-	-	-	-	-	72	102	71	-	-	-	-	-	-
DDE 4.0%	-	-	-	3	45	7	-	-	-	18	90	20	-	-	-	145	-	-
dieldrin 4.0%	-	-	-	21	52	40	-	-	-	22	50	44	-	-	-	186	-	-

D = number dead      T = total exposed      % = percentage mortality

Fig.27

Resistance spectra of *A.hyrcaus* from Chukurova Plain, Turkey.



### DISCUSSION

The present study has demonstrated the necessity and the importance of both laboratory as well as field observations for studying the varying aspects of insecticide resistance. While laboratory studies have their advantages in providing standardisation of living material and test conditions, they also have their limitations in the genetic variability available in populations usually derived from a few individuals and subjected to a degree of inbreeding in closed self-perpetuating populations. Laboratory populations, unless continually being added to from the field, are thus not necessarily, entirely representative of natural (field) ones. This may assume considerable importance where multiple resistances are being studied. When such studies are made without the possibilities of renewal from field populations, all possible attempts need to be made to retain the already available genetic material.

With regard to those species which are not easily maintained and where large samples are necessary, as in the studies of resistance spectra, field studies are of considerable value at least in terms of saving time and effort, apart from any other considerations. In these field situations problems are encountered in standardising both test conditions and test material. In such cases comparison of different populations in comparable field conditions may assist in the assessment of the resistance situation. Such comparisons are advantageous when known susceptible populations of certain species may not be available. Thus in A. sacharovi populations from the Chukurova Plains of Turkey the survivors of exposures to discriminating dosages of most insecticides were established as representing resistant individuals, by comparison with other field populations of the same species from different parts of Greece. The latter showed either susceptibility or lower

levels of resistance to the compounds tested. However in these situations variations in temperature should be taken into consideration when interpreting the results.

Laboratory studies are particularly useful with regard to genetic and biochemical studies and the establishment of discriminating dosages of insecticides. In the present study, the majority of the populations being difficult to maintain (some of them even not mating in cages), the observations were restricted to genetic studies and to obtaining the baseline data in the hope of continuing the studies in the field where sufficient material could be obtained. This was not possible in certain instances however, as in the case of A. culicifacies. This led to considerable variations in the sample size of the different populations studied and to observations being incomplete and not altogether conclusive.

The discriminating dosages established in the present study for the detection of resistance in populations were based on limited tests of presumably susceptible populations, mainly the ST/15 strain of A. stephensi, and the CUL/SRI strain of A. culicifacies. However, continued testing of these populations as well as others have quite often revealed occasional survivors to these dosages. Failure to receive a lethal dose of the respective insecticides by resting on the untreated netting of the exposure tubes, reduced effectiveness of the impregnated papers (through constant use or ageing) or even the possible effect of fluctuating test temperatures, to which some species such as A. stephensi were found to be highly sensitive, are all possibilities that could account for such survivors. However, the possible existence of rare resistant individuals cannot be ruled out. With certain populations such as the ST/ROK and ST/BAR strains of A. stephensi, A. sacharovi and A. hvrcanus, such survivors were actually indicated as representing resistant genotypes. With others such as the ST/15

strain of A. stephensi and the CUL/SRI strain of A. culicifacies, all presumably susceptible to the insecticides under consideration, the significance of the survivors was not determined.

In the use of the discriminating dosages, the time of exposure to the relevant insecticides is usually standardised at one hour. However, it has been reported, (Technical Report Series 585, W.H.O. 1976) as was also observed in the present study, that with some insecticides such as fenitrothion, the established 1.0% concentration at one hour exposure was insufficient to kill presumed susceptibles of A. sacharovi and A. maculipennis. For most other species such as A. stephensi, A. culicifacies and A. albimanus, the 1.0% fenitrothion for one hour exposure was sufficient for this effect. The 0.001% decamethrin used in the present study was found to be insufficient to discriminate the susceptible genotypes. This however was later rectified so that 0.0025% for one hour exposure is now the recommended dosage. In addition, studies have also revealed that dosages of 2.5% fenthion for one hour and 0.1% propoxur for one hour, which applied very well to such species as A. maculipennis and A. sacharovi, were higher than necessary for most other species e.g. A. stephensi and A. culicifacies. In the latter it was evident that on a set of recently impregnated papers a 15 or 30 minutes exposure with the relevant dosages could produce a 100% mortality. Similarly, the 5.0% malathion for one hour exposure may be higher than necessary for many species. A. sacharovi and A. maculipennis populations from Greece were killed by a 30 minute exposure to 5.0% malathion while past field testing of A. stephensi and A. culicifacies with 3.2% malathion for one hour has produced complete mortalities.

It has been observed that recently impregnated papers or those newly unpacked are more effective than the ones used for a period of time,

for obvious reasons. However this fact in addition to the use of too high a dose may conceal resistance in the very early stages of its development. This is exemplified in the present study in the situation where in the A. stephensi ST/ROK wild population, a recently prepared set of fenitrothion papers gave a 100% mortality. On this basis the population was considered susceptible to this insecticide. However, in the same population, the presence of individuals resistant to this insecticide was revealed on a set of used papers which had also produced a 100% mortality of the susceptible ST/15 population. The prevalence of these resistance factors in this population was subsequently confirmed following selections with this insecticide (Table 14 and fig. 18 for ST/ROK and Table 14 and fig. 17 for SMB6, EM3.16 and E136). Therefore the state of the impregnated papers in the detection of resistance factors should always be taken into consideration.

Species variation in the discriminating dosages do not appear to be associated with variation in the size of the mosquitoes concerned, since with some compounds such as malathion the larger-sized A. sacharovi. and A. maculipennis needed a much lower dose to give a complete kill than the smaller-sized A. superpictus, A. culicifacies, and A. stephensi. The opposite effect was observed with propoxur, fenthion, and fenitrothion with these same species. It may be that resistance factors for some insecticides are more prevalent in some species than in others. Therefore a few of these could remain undetected in strains used to establish the discriminating dosages, since the full susceptibility of these is rarely confirmed. This could to some extent account for variations in the so-called discriminating dosages necessary and also for the more common occurrence of resistances shown in these species towards the same insecticides where resistances could eventually be demonstrated. Thus in species such as A. sacharovi and A. maculipennis

where the "discriminating" dosages for fenitrothion, propoxur and fenthion are higher than those for A. culicifacies and A. stephensi the frequency and levels of resistance are also generally high.

Even though the resistance spectra of a number of populations have been studied, these on their own have not contributed to any extent in the determination of the nature of the mechanisms involved. Where single resistances are concerned the analogues which would have helped in this identity were not available for testing. In those which had already developed multiple resistance, the interactions of the mechanisms themselves would have concealed the diagnostic characteristics of the individual resistances. Wherever feasible, synergists were used to help in the identity of the mechanisms concerned. Here too, the observations may be incomplete in view of the fact that in most instances either the test material or the synergists were not available at one and the same time.

Most of the mosquito populations studied were in varying stages of heterogeneity for their resistance factors. Variations also existed in test material and in test conditions. In addition, in the use of the contact testing method, the quantities of insecticides picked up by individual mosquitoes could vary considerably. Therefore, although quantitative estimations were attempted, when comparing populations, the indices used were only approximations for gaining an insight into the nature of the resistance patterns in existence.

Comparison of the resistance spectra of the different populations has revealed a diversity in their response to the different insecticides, both within species as well as between different species (i.e. intraspecific as well as interspecific differences). (Table 51)

In general populations varied from those:

- (a) apparently susceptible to all (fig. 24 (PALB))
- (b) resistant to only DDT (fig. 2 (CUL/SRL))
- (c) resistant to DDT and dieldrin
- (d) resistant to organochlorines and OP's (fig. 2 (CUL/IND))
- (e) resistant to organochlorines, OP's and carbamates (fig. 24 FERN/RR)
- (f) resistant to organochlorines, OP's, carbamates and the pyrethroids (Fig. 28)

Thus among the many populations of the 7 species of anophelines studied, with the exception of A. superpictus from Greece (where only a very limited sample from a single locality was tested with only a few insecticides) all have shown either one or more populations with already developed multiple resistance or the potential for such development (through laboratory selection).

In general it appears that the potential for the development of resistance towards most compounds can exist in most species and the variations observed may reflect the different types of selection pressures they have been subjected to. However, in most populations studied the information regarding the exact nature, extent and the types of insecticides to which they have been exposed is not available for any proper assessment of the possible impact of these in the development of the resistances observed. But insecticides of different types have been used either for agricultural or public health purposes. Any attempts made to deduce the possible selections are therefore only speculative.

Georghiou (1975b) attaches great importance to the indirect selection pressure by agricultural pesticides in the development of resistance in mosquitoes especially through aerial applications. The multiple resistance development in A. albimanus from El Salvador was attributed to this as well as use of insecticides in public health. Ramsdale (1975) similarly drew attention to the contribution by agricultural usage of insecticides in hastening the development of DDT



resistance in A. sacharovi in the Adana area of Turkey. A report by Gangoli (1975)\* has detailed the wide range of insecticides applied in the cotton growing areas in the Chukurova Plain of Turkey. It appears that these may account for the broad spectrum of OP and carbamate resistance now exhibited in A. sacharovi, A. maculipennis and A. hyrcanus from these areas.

As far as A. culicifacies from Sri Lanka is concerned, although a variety of insecticides are known to be used in agriculture, the extent is by no means comparable to that in Turkey. Further, aerial applications are not made. The only insecticide to which this species has so far shown resistance is DDT, which is the insecticide used until recently for the control of this vector mosquito in the malaria control programme. It seems likely therefore that the extent of selection by agricultural pesticides has been comparatively limited. The endophilic tendency of this species favours selection by the residual insecticides used for spraying houses while the usual type of breeding place, river beds, may not be subjected to great contamination from agricultural pesticides applied some distance away. However development of resistance, particularly with OP's, is known to be prolonged in the initial stages and therefore it is not possible to say with certainty that agricultural pesticides will not contribute to the selection. It seems probable that the process is expedited by its use in malaria control as is to be expected. Further, in Sri Lanka, it cannot be foreseen if this lack or the limited extent of selection by the agricultural pesticides so far seen will continue. Recent developments in agricultural strategy following diversions of one of the major rivers, resulting in changes in the irrigation structure, could have an impact both in terms of breeding places as well as in the agricultural usage of insecticides.

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\* Ref. Report by D. S.D. Gangoli (TUR/CEPOO2, UNDP/TUR/75/023).

Similarly, in the appearance of malathion resistance in A. culicifacies in India, A. stephensi in Iran, and A. arabiensis in Sudan, although any contribution from agricultural pesticides is not contradicted, it appears that the use of this pesticide in the malaria control programme has again hastened the speed of its development.

It seems appropriate to consider the extent to which factors other than insecticide selection pressure may also account for the variations in patterns of resistances observed among the multiresistant populations themselves. Thus the A. culicifacies CUL/IND from the Maharashtra State, India and the laboratory selected SMB6, EM3.16 and E136 populations of A. stephensi from Iran all showed a broad spectrum of OP resistance with high susceptibility to the carbamates. Although the OP resistant A. hyrcanus populations from the Chukurova Plain of Turkey were more susceptible to the carbamates, those of A. sacharovi and A. maculipennis from the same area and A. albimanus from El Salvador, showed both OP and carbamate resistance. The high fenitrothion resistance shown by the three species A. sacharovi, A. maculipennis and A. hyrcanus, all from the Chukurova Plain of Turkey was not encountered in any of the other species so far studied. Species also differed in the nature of their response to malathion and fenitrothion. In A. culicifacies from India, A. stephensi from Iran, and A. arabiensis from Sudan, malathion resistance appeared to be more common, and the levels of resistance generally high. In addition this resistance could exist on its own or in association with fenitrothion resistance as was demonstrated in A. culicifacies, and A. stephensi. However, there was no single instance of a report so far in these species, of resistance to fenitrothion in the absence of that towards malathion (Tables 49 and 50, Fig. 29). In contrast, in the three species from the Chukurova Plain of Turkey, the fenitrothion resistance was more common, and the levels probably higher, and usually not accompanied by malathion resistance (Tables 49 and 50 and Fig. 30)

Table 49. Percentage mortalities in field populations of *A. colicincta* in various localities in the Maharashtra State and Selected States of India in 1976 and 1977 after exposure to the discriminating dosages of malathion and Fenitrothion (figures in parentheses represent dates of testing)

Locality	Malathion	Fenitrothion
<u>Bangalore - India</u>		
Somena Hallay	100 (1977)	100 (1977)
Amrutur	100 (1977)	100 (1977)
Hediagehally	100 (1977)	100 (1977)
Solekere	100 (1977)	100 (1977)
Sidlaghatta	100 (1977)	100 (1977)
Lingadha Halli	100 (1977)	100 (1977)
Goa (Sanguem)	93 (1977)	100 (1977)
<u>Maharashtra State - India</u>		
Asta Tai Kansot	83 (8.9.76)	80 (8.9.76)
Anturli	67 (19.8.76)	100 (22.9.76)
Jamner	48 (20.8.76)	100 (22.9.76)
Pimpalkotha	55 (7.10.76)	100 (7.10.76)
Akulhede	83 (11.10.76)	100 (11.10.76)
Rumbudi	85 (29.10.76)	100 (29.10.76)
<u>Gujarat State</u>		
Shivad	100 (17.4.76)	-
	100 (18.6.76)	-
	97 (22.8.76)	-
	91 (17.12.76)	74 (17.12.76)
Gandera	25 (24.8.76)	100 (24.8.76)
Sasan	94 (24.8.76)	83 (22.12.76)
Asta	83 (8.9.76)	80 (8.9.76)
Delvada	90 (16.9.76)	100 (16.9.76)
Ihat-Taj	22 (18.9.76)	100 (18.9.76)
Butvada-Taivad	30 (20.9.76)	88 (20.9.76)
Omedpura	27 (18.11.76)	62 (23.12.76)
Panchwada	86 (28.5.76)	-
	28 (21.12.76)	58 (21.12.76)
	52 (4/77)	58 (4/77)
Sasam	87 (22.12.76)	83 (22.12.76)
Umadpura	33 (-/12/76)	-
	53 (24.4.77)	62 (24.4.77)
Chikatiya	32 (29.1.77)	48 (29.1.77)
Jabirgan	82 (18.1.77)	52 (18.1.77)
Pal & Chanishera	70 (21.1.77)	31 (20.1.77)

\*Data made available to W.H.O. by N.M.E.P. (India).

Table 50. Percentage mortalities in various laboratory and field populations of different anopheline species after exposure to the discriminating dosages of malathion and fenitrothion

Locality	<u>A. stephensi</u>	Malathion	Fenitrothion
Laboratory:-			
ST/15 (susceptible) from Delhi, India	98	100	
ST/245 - from Bangalore, India	99	98	
ST/ROK (unselected) from Iran	99	100	
SM 35 (malathion selected)	11	97	
SM 35 malathion - fenitrothion selected	6	77	
EM 3.16 malathion - fenitrothion selected	2	83	
E 136 malathion - fenitrothion selected	0	79	
<u>A. gacharovi</u>			
Laboratory tests:-			
Soysalli	100	88	
fenthion selected	100	0	
Field tests:-			
from Greece:			
Sufli } 19° - 25°C	100	75	
Poros }	100	31	
Anthili }	100	25	
from Turkey:			
Asagi Kulak } 31° - 33.5°C	100	33	
Kucuk Karatas }	99	33	
Tabaklar }	100	9	
Asagi Kulak } 21° - 27°C	90	2	
Kucuk Karatas }	90	2	
<u>A. maculipennis</u>			
from Greece:			
Sufli } 19° - 25° C	100	84	
Poros }	100	64	
<u>A. hyrcanus</u>			
from Turkey:			
Laboratory tests:			
Unselected	90	10	
fenitrothion selected	77	7	
fenthion selected	85	5	
Field tests:			
from Chukurova Plain:	100	53	
<u>A. arabiensis</u> (from WHO)			
from Sudan:			
Field tests:			
Gezira	-	100 (18.10.73)	
Tabgar	100 (20.5.75)	100 (20.5.75)	
Barkar	90 (-/11/77)	100 (-/11/77)	
Laboratory test (Akood personal communication)			
Gezira	39	100	
<u>A. albimanus</u>			
Laboratory tests:			
PALE	100	99	
PERNS/RR	39	14	

Fig.29

Percentage mortalities resulting from exposures to the discriminating dosages of malathion and fenitrothion to various field populations of *A.culicifacies* from the Maharashtra and Gujarat states of India.

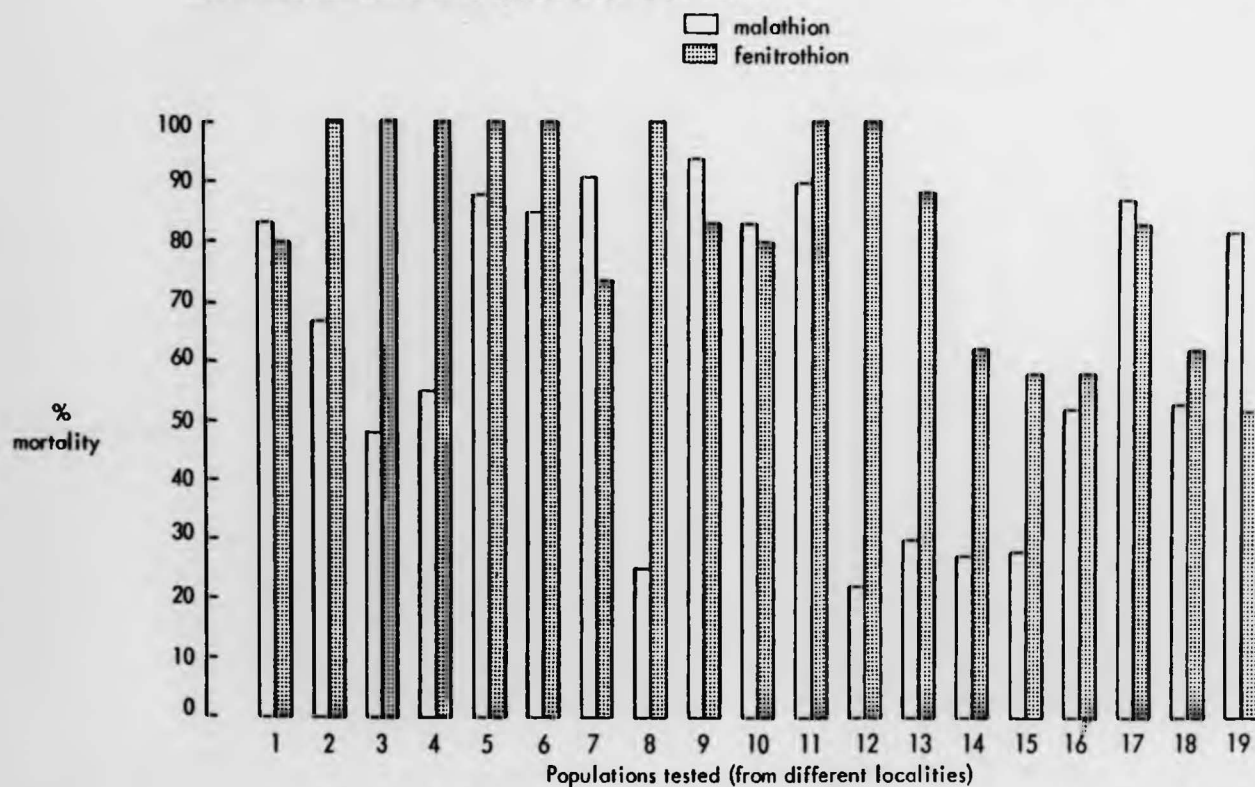
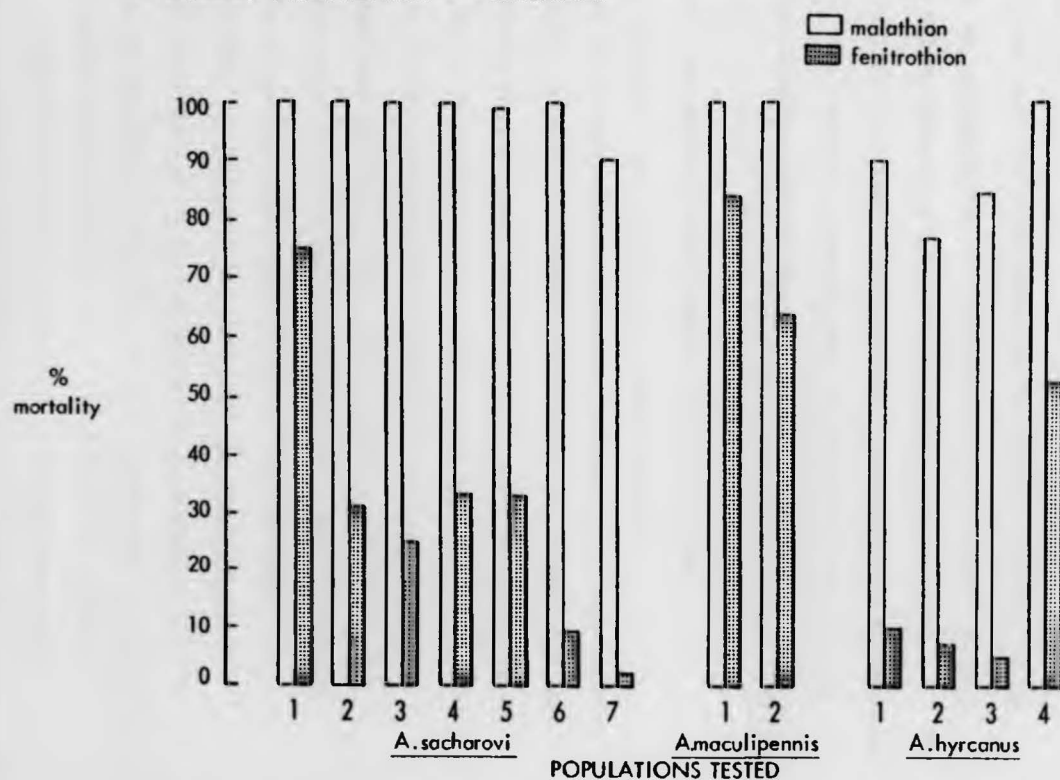


Fig.30

Percentage mortalities resulting from exposures to the discriminating dosages of malathion and fenitrothion to various populations of A.sacharovi, A.maculipennis and A.hyrcaus.



Further, resistance to the latter appeared to be rare and in the few instances encountered, can be considered to be accompanied by the fenitrothion resistance.

Even though the resistance to malathion in A. culicifacies and A. stephensi can be attributed to the selection pressure exerted by this insecticide used for house spraying for malaria control, it is known that both A. albimanus and the species from the Chukurova Plain of Turkey were all subjected to intensive selection pressure from malathion and fenitrothion used in agriculture. Therefore the differences in the nature of the response, particularly to malathion (very low in the Turkish populations), could be due to the effect of factors other than the selection pressure. Since the same explanation seems to be applicable to all the species in this region, it may be that some particular factor in the Turkish environment affects the predominance of the malathion resistance factors. It is more likely that this variation is in the carboxylesterase mechanism rather than any other, since malathion resistance has not been recorded so far to exist on its own in any of these populations. Such geographical variation in the predominance of the carboxylesterase mechanism has been suggested to occur in houseflies (Sawicki 1975a). Here malathion resistance due to the carboxylesterase mechanism was considered less common in Denmark, while in the USA it was primarily attributed to this mechanism.

The absence of carbamate resistance in A. culicifacies and A. stephensi and its presence in A. albimanus and A. sacharovi may be explained solely on the use of this type of insecticide for agricultural and for public health purposes where the latter species are found. The potential for the development of carbamate resistance has been demonstrated in A. stephensi in the laboratory (figs. 9 and 18). Carbamate resistance in A. sacharovi but not in A. hyrcanus even though from

the same agricultural area of Turkey, may reflect a species difference in the presence or absence of the resistant factors or a difference in their predominance. The occurrence of fenthion resistance in both A. sacharovi and A. hyrcanus and its absence in A. albimanus might be due to differences in the types of insecticides used in agriculture in the two countries concerned unless here again geographical variations exist with regard to the predominance or existence of the resistant factors involved. In a strain of Culex quinquefasciatus from Burma the fenthion resistance was attributed to a hydrolytic esterase whereas in houseflies, involvement of mfo's has been suggested (Keiding 1975). In A. sacharovi, although the nature of resistance to this insecticide was not specifically investigated, synergists showed that the fenitrothion resistance was associated with both mfo's and hydrolytic esterases. Whether the same enzyme systems suggested for fenitrothion resistance are also contributing to fenthion resistance in the same population is not known however. The resistances to both insecticides are high, however, in this species.

With regard to the resistance to pyrethroids, since these insecticides are claimed never to have been used in Turkey, it might be attributable to a possible cross resistance imparted by the factors involved in the DDT resistance in A. sacharovi. Here too, as indicated earlier under the Results section, apart from the involvement of both mfo's and DDT-ase suggested by synergist evidence, there were indications of additional factors, possibly a knockdown resistance mechanism. The latter as well as the mfo's are known to contribute to pyrethroid resistance in houseflies. In the A. culicifacies CUL/SRL from Sri Lanka and the ST/BAR and STR/DDT strains of A. stephensi from India and Iran respectively, on the other hand, the DDT resistance was demonstrated to be primarily due to the DDT-ase mechanism. Here again even though a probability of either a species or a geographical difference could be considered, in the absence of comparable histories



of selection in the populations concerned, this is only a speculation. The importance of resistance factors additional to DDT-ase in A. sacharovi from the same area of Turkey has also been suggested by Perry (1959).

It should be pointed out that although A. sacharovi from the Lamia Plain of Greece showed DDT resistance, A. superpictus also from the same area was susceptible, in spite of the fact that both may have been subjected to the selection pressure of DDT as used in malaria control.

It may not be a coincidence that those species which breed in swampy areas with a lot of vegetation, have shown resistances dependent on more generalised resistant factors, such as the mfo<sup>1</sup>, whereas those species breeding in clear open water without much vegetation tend to show resistance dependent on the more specific mechanisms.

As pointed out earlier, because most populations are heterogeneous for the resistance factors, the levels of the resistances determined are only approximations. However, in the two populations A. culicifacies CUL/IND and A. stephensi E136, both homozygous for malathion resistance, the degree of resistance to this insecticide differed considerably. In A. culicifacies, the involvement of two factors in this resistance, the carboxylesterase, and a generalised mfo mechanism was suggested earlier, both being in the homozygous condition. The very high tolerances may be a combined effect of these two mechanisms. In A. stephensi on the other hand, although some survivors to fenitrothion and other OP<sup>1</sup> occurred in this population the resistance to which was attributed to the generalised mechanism, also contributing to malathion resistance, the proportion of these survivors was relatively low. Therefore, most of the resistance to malathion in this population may be more attributable to the carboxylesterase mechanism which may have its own limited detoxication capacity. This may also account for the

failure to enhance the level of malathion resistance in the SM35 population of A. stephensi in spite of continued efforts, as only the carboxylesterase mechanism was demonstrated in this population by the use of synergists. On the other hand in the three populations SMB6, EM3.16 and E136 of A. stephensi all with increased proportions of fenitrothion resistant individuals (presumably those with the generalised defence mechanism) the level of malathion resistance was high compared with the SM35. This again lends support to the idea that this generalised mechanism may be contributing to increased levels of malathion resistance. Similarly the possibility exists that in the attempts to select a population with a high level of malathion resistance, those individuals that survived 3 and 4 hours exposures of 5% malathion may have represented individuals having both the carboxylesterase and the generalised mechanism. The differences in the levels of resistances in the two populations CUL/IND and E136 were also well demonstrated in their heterozygotes. While in A. culicifacies this was almost completely dominant (an identical response in the resistant parent and heterozygote at the discriminating dosage) in A. stephensi the heterozygote proved to be intermediate between the two parent populations in its insecticide tolerance.

Of serious practical significance is the resistance shown by a number of populations towards malathion, fenitrothion, chlorphoxim, phoxim, iodofenphos and pirimiphos methyl, all considered as potential alternatives to DDT in malaria control. Figs. 31-35 compare the responses of the relevant susceptible and resistant populations of the species studied to these insecticides. As mentioned earlier in A. albimanus and A. sacharovi the development of this resistance was attributed to the use of agricultural pesticides as well as to those used for public health purposes. In A. culicifacies and A. stephensi even though agricultural pesticides may have contributed to some extent

Fig.31

Proportion of individuals surviving the discriminating dosage of malathion in different populations of anophelines.

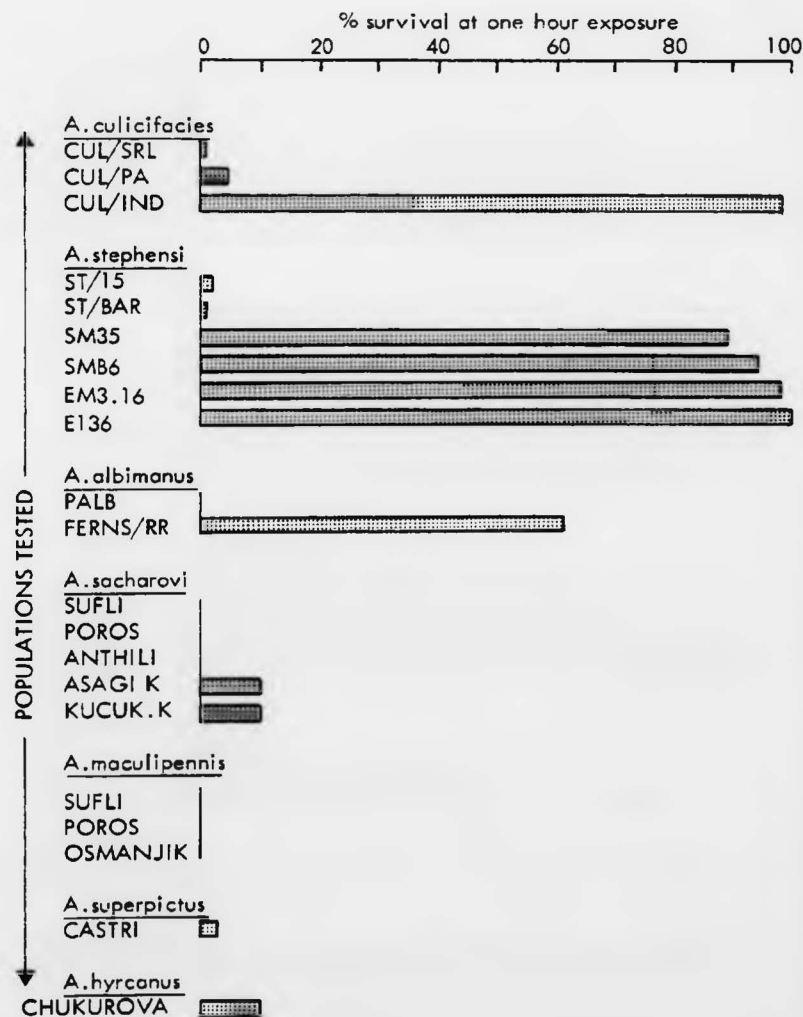


Fig.32

Proportion of individuals surviving the discriminating dosages of fenitrothion in different populations of anophelines.

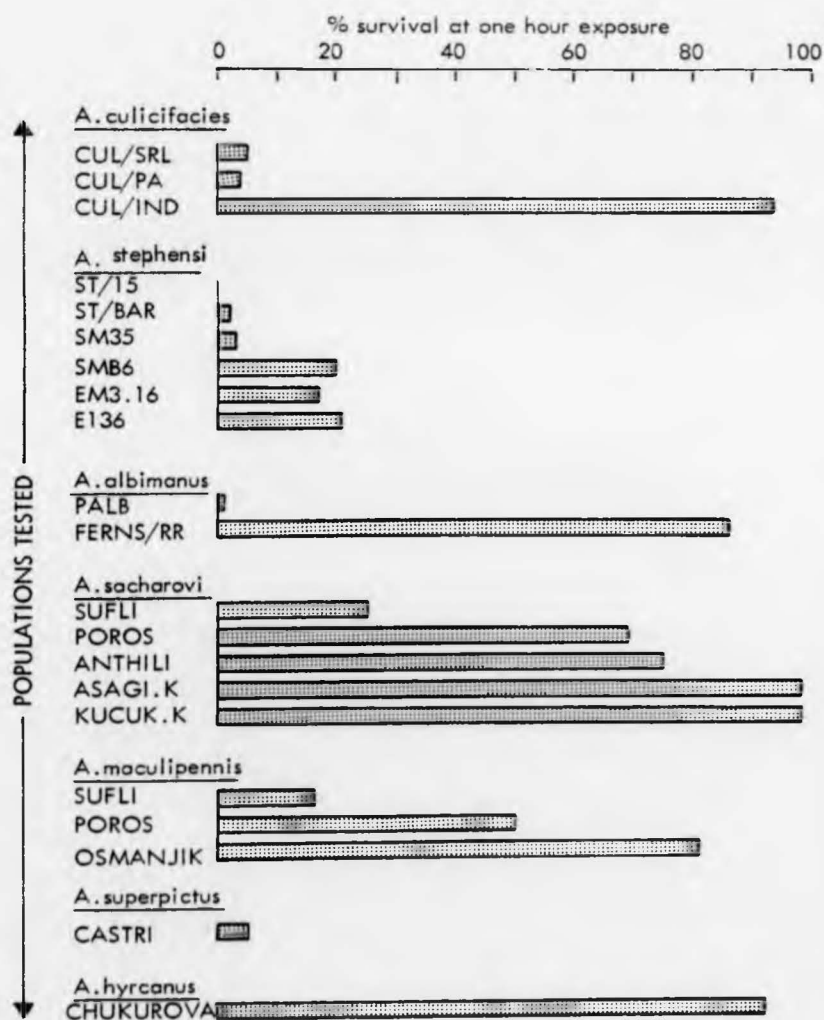


Fig.33

Proportion of individuals surviving the discriminating dosage of pirimiphos methyl in different populations of anophelines.

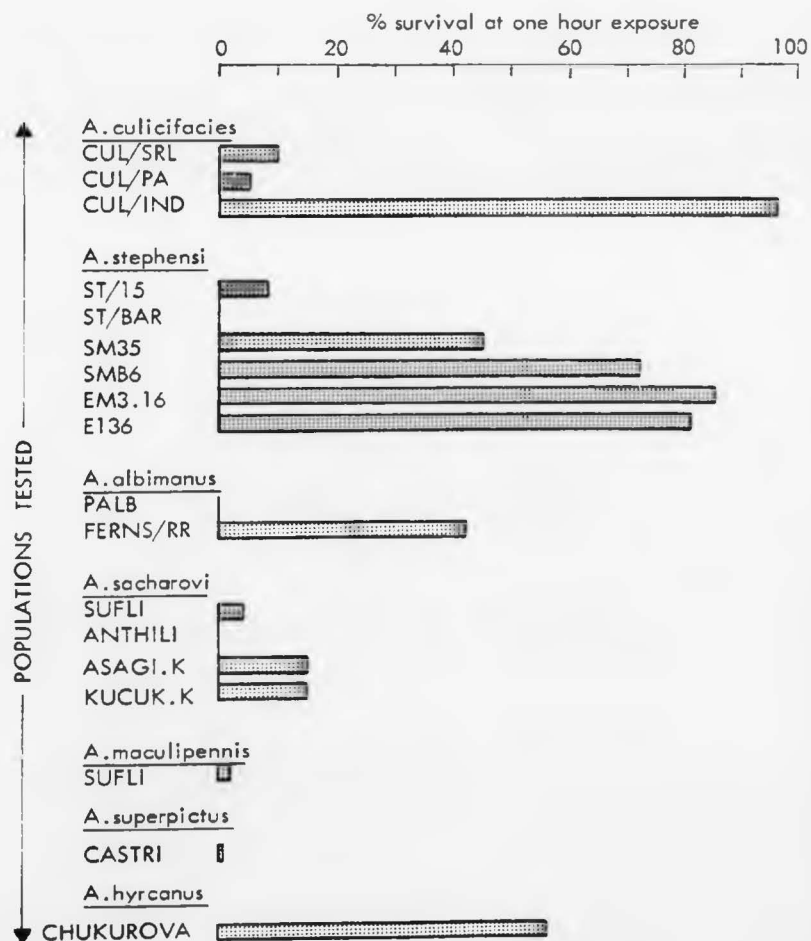


Fig.34

Proportion of individuals surviving the discriminating dosage of chlorphoxim in different populations of anophelines.

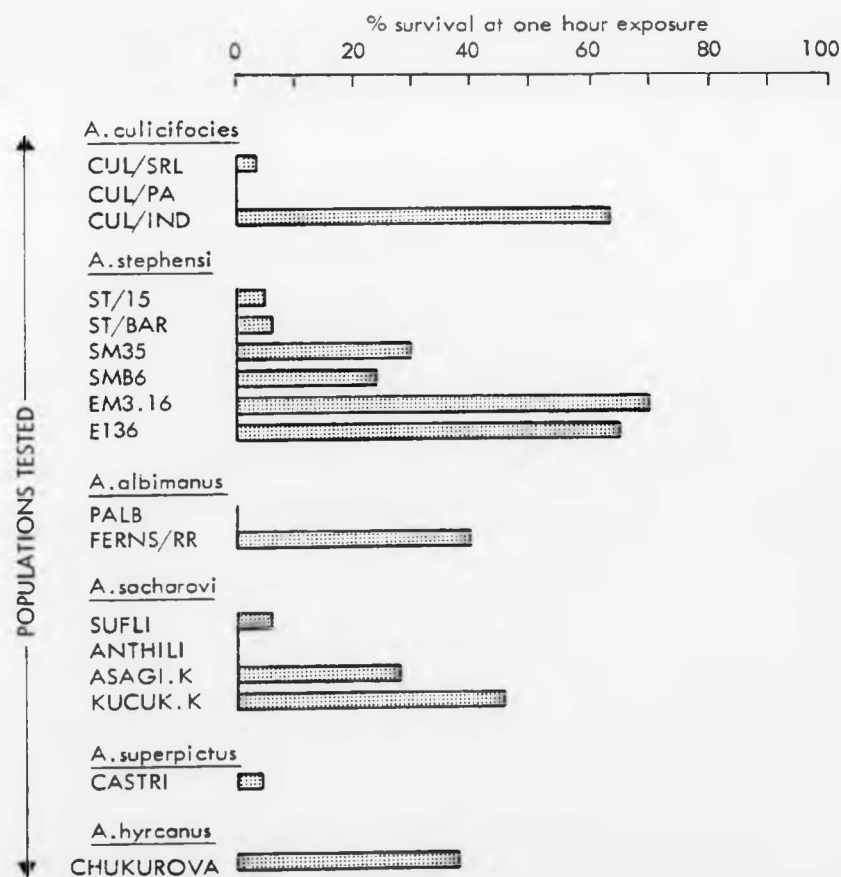
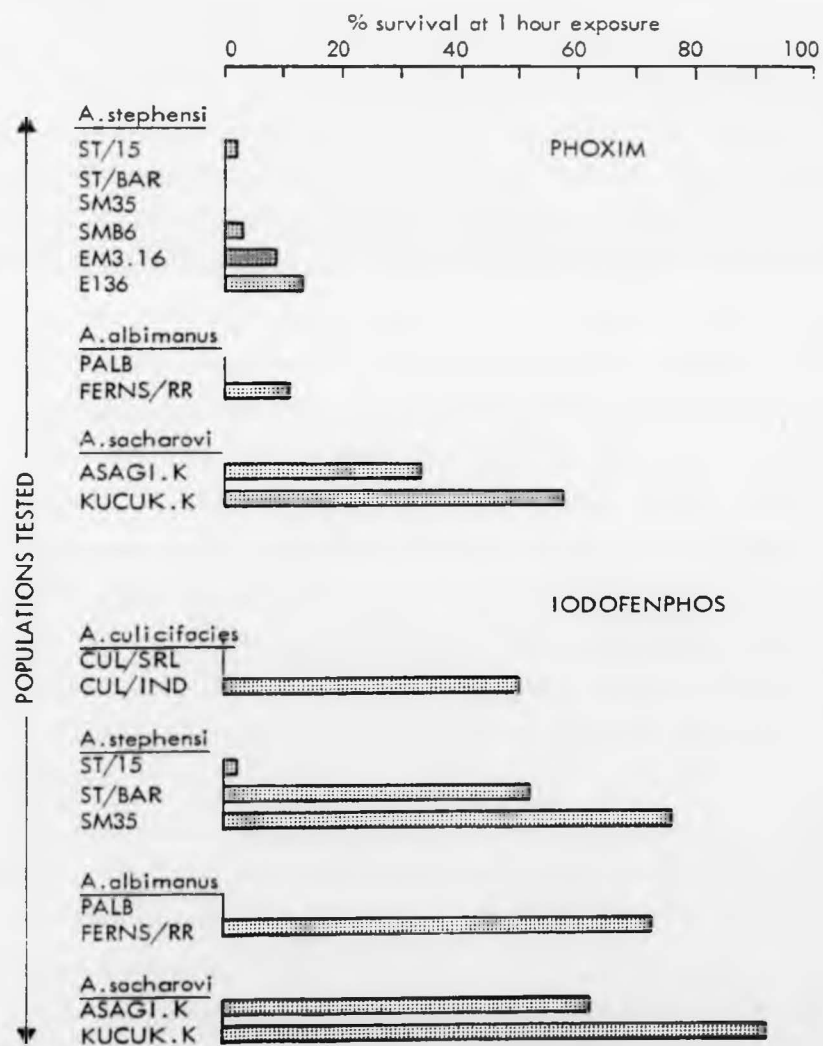


Fig.35

Proportion of individuals surviving the discriminating dosages of phoxim and iodofenphos in different populations of anophelines.



(detailed information on insecticide usage in agriculture is not known), it appears that the use of malathion in the malaria control programmes has hastened the resistance development to this insecticide. It therefore seems important to consider, if the latter has also contributed to any extent to the development of multiple resistance in the A. culicifacies (CUL/IND) population from India. As far as this is concerned, the only population available for study being difficult to maintain, it was not possible to investigate the nature of its multiple resistance in a manner comparable to the approaches adopted by Georgiou (1971) and Sawicki (1973) in houseflies. In the latter they isolated the individual resistance mechanisms, studied their characteristics, and determined the nature of their interactions by resynthesising the multiple resistances. Therefore in the present study, disregarding any possibilities of species variations, certain observations on the two species, A. culicifacies from India and A. stephensi from Iran have been combined (and correlated) in an attempt to understand the nature and possible causes of the multiple resistance observed in the former. In addition to the information from genetic studies, the use of synergists, and resistance spectra determination in both species, certain field data were also utilised concerning A. culicifacies. With A. stephensi on the other hand, being easily reared, the trends observed during the attempts to develop a multiple resistant population in the laboratory was taken into consideration.

The field data on A. culicifacies from Maharashtra State, India, on A. stephensi from Iran, and A. arabiensis from Sudan, in addition to the laboratory observations of the malathion selected SM35 population of A. stephensi also from Iran, all indicate the initial response to malathion selection pressure to be the selection of the specific carboxylesterase mechanism. This was evident from synergism and cross resistance studies which showed no cross resistance to fenitrothion and

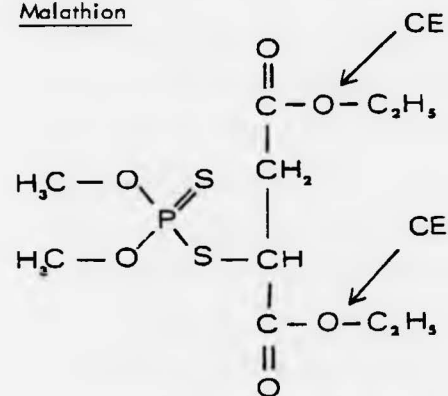
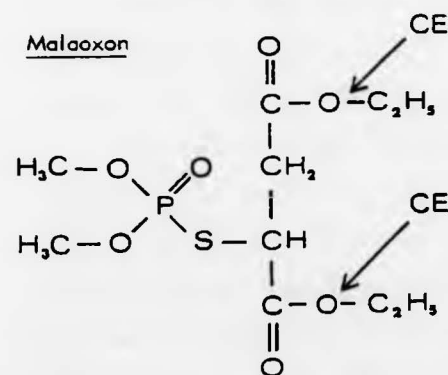
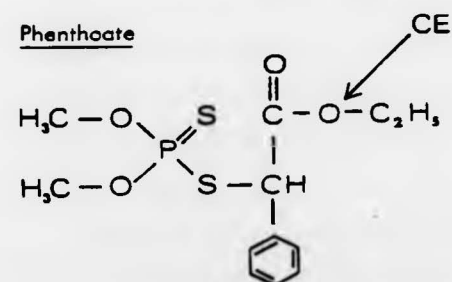


other potential alternatives having no carboxylester bonds. Fig. 36 illustrates some of these compounds showing either the presence or the absence of carboxylester bonds. This explains the lack of resistance to fenitrothion so far in A. arabiensis (Akood, personal communication) and in the field populations of A. stephensi. In the multiresistant population of A. culicifacies the backcross results did not unequivocally establish the number of genetic factors involved in malathion resistance. However, a possible involvement of carboxylesterases as a mechanism of this resistance was indicated by synergism with TPP. This hypothesis may be further supported from the indirect evidence from field data received from areas from which the multiresistant CUL/IND population was received (Data provided to the World Health Organization by the National Malaria Eradication Programme of India). Table 49 indicates the results of susceptibility tests using malathion and fenitrothion discriminating dosages on samples of populations made apparently at earlier stages of resistance development (available only as far back as 1976). Out of samples from 6 localities in the Maharashtra state, only one shows resistance towards both insecticides, whereas in the others malathion resistance was unaccompanied by resistance to fenitrothion. From Gujarat State, in which 14 localities have been sampled for response to the 2 insecticides 3 have shown resistance to malathion, with full susceptibility to fenitrothion, while the rest show resistance to both insecticides. Therefore malathion resistance in the absence of that towards fenitrothion can be assumed to be a result of an initial or independent selection of the malathion specific carboxylesterase mechanism in the population, which will not impart cross resistance to fenitrothion or other compounds lacking the carboxylester bonds.

In the malathion selected SM 35 population of A. stephensi where only the carboxylesterase mechanism was indicated cross resistance

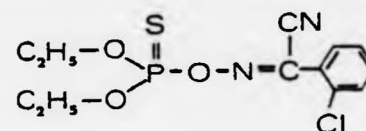
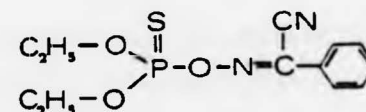
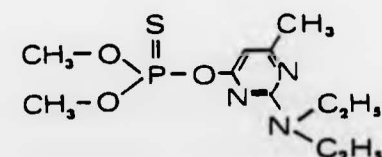
Fig. 36

Compounds with carboxylester bonds

MalathionMalaoxonPhenthoate

CE = carboxylester bond

Compounds without carboxylester bonds

ChlorphoximPhoximPirimiphos-methyl

to fenitrothion and other OP's such as chlorphoxim, phoxim and pirimiphos methyl, could not be demonstrated. Subsequent selection of this population with fenitrothion, however, showed a tendency for a concurrent increase in tolerances to these compounds (Fig. 17), suggesting a possible relationship between these resistances and resistance to fenitrothion. In addition, as pointed out earlier, the three lines of the malathion-fenitrothion selected populations, SMB6, EM3.16 and E136, all with increased proportions of fenitrothion resistant individuals also showed an increase in the level of malathion resistance, when compared to the SM35 selected only with malathion. In this instance, unless the expression of the carboxylesterase factor itself is greater in the presence of other resistance factors, the possibility exists that a common resistant factor which may be conferring resistance to fenitrothion and the other OP's referred to earlier may also be contributing to malathion resistance to some extent.

The pattern of OP resistance seen in the malathion-fenitrothion selected populations of A. stephensi seems to be comparable to that seen in the multiple resistant populations of A. culicifacies from India and A. albimanus FERN/RR population. The two latter populations showed a response to malathion, following pretreatment with PB, different from that shown by the SM35 population of A. stephensi (where only the carboxylesterase was demonstrated), on which basis a possible involvement of an mfo mechanism in malathion resistance was suggested for the two former populations.

Overall then it seems possible that in certain populations individuals could exist with only the carboxylesterase mechanism, only the generalised mechanism or with both. The eventual stabilisation of any of these resistances in populations will be dependent on the fitness of the respective genotypes, when on their own as well as when they are in combination. As far as A. culicifacies, A. stephensi and

A. arabiensis are concerned the reported existence of malathion resistance in the absence of fenitrothion resistance, may suggest the relative fitness of the genotype with only the specific carboxylesterase mechanism. Multiple resistances involving malathion and other OP's shown in A. culicifacies, A. albimanus and A. stephensi, where the carboxylesterase mechanism could be demonstrated may suggest the fitness of the genotypes with both resistant factors. In these species there is no evidence as yet to establish the fitness of the genotypes with only the generalised mechanism.

In A. culicifacies from Maharashtra State, India, where records of the response to both malathion and fenitrothion are available from 20 localities (Fig. 29) malathion resistance was found to exist either on its own or in combination with fenitrothion resistance. The lack of any reports so far of any fenitrothion resistance without the concurrent existence of resistance to malathion may be suggestive of two possibilities. The generalised mechanism on its own is able to confer sufficient resistance even to malathion, or this genotype cannot stabilise itself successfully in the absence of the malathion specific carboxylesterase mechanism. It should be pointed out that lack of positive evidence to confirm this in A. culicifacies could be purely a consequence of insufficient sampling. Similarly with A. stephensi the failure to establish a fenitrothion resistant population without malathion resistance in the laboratory may be the result of insufficient attempts and the type of selection procedure adopted not being suitable.

Although the extent of the response to malathion and fenitrothion in A. sacharovi, A. maculipennis and A. hyrcanus may differ from those in other species (in that fenitrothion resistance was common and high and malathion resistance rare), the lower, but more or less equal levels of resistance shown to the other OP's referred to, combined with the

limited level of resistance to malathion, may all suggest a generalised mechanism like that in A. culicifacies. The high resistance to fenitrothion shown by the Mediterranean species might be attributable to a hydrolytic esterase as well as mfo involvement. This was suggested from synergist evidence in A. sacharovi but comparable observations are lacking for all other OP's and for all other species. What is required is to establish if the limited levels of resistances to malathion and other OP's such as chlorphoxin, phoxim, and pirimiphos methyl seen here are a reflection of a poor fitness (lower adaptability) of the genotypes with the generalised resistance factor when on its own (in the absence of the carboxylesterase or other resistance mechanisms), an effect of the types of the selection pressures, or due simply to the rare occurrence of this resistance factor itself in the population. The limited levels of the malathion resistance suggest that the more important carboxylesterase mechanism for malathion resistance may be rarer in these populations than in A. culicifacies, A. stephensi, and A. arabiensis where the specific mechanism appears to be predominant.

It appears that in species such as A. culicifacies, A. stephensi and A. arabiensis the eventual outcome of malathion pressure, apart from the inevitable development of resistance to this insecticide itself, is to produce a multiple resistant population provided the generalised resistance factor is in existence. The speed of development will obviously be determined by the frequency of the resistance factors.

Assuming that the predominance of the carboxylesterase mechanism in A. culicifacies, A. stephensi and A. arabiensis is true, then this fact could be taken advantage of in an attempt to extend the operational life of the few insecticides available (such as malathion and fenitrothion) in malaria control, by using the optimal sequence to delay the

resistance development, as was suggested by Sawicki (1975b). Thus in populations such as A. culicifacies and A. stephensi in which the specific malathion resistant mechanism appears to be common, the initial use of this insecticide would select genotypes with the specific mechanism, as well as those with both carboxylesterase and the generalised mechanism. Selection of the generalised mechanism on its own will depend on whether this mechanism confers resistance to malathion. On the other hand, if fenitrothion is used prior to malathion, then at least the selection of those genotypes with only the specific mechanism may be avoided, thereby slowing down the development of malathion resistance to some extent. Further in the event that the mfo factor is poor in its adaptability in the absence of the specific mechanism (an assumption needing confirmation) the development of resistance could be further delayed. These are however only theoretical assumptions needing confirmation. Field trials with comparable independent selection with the two insecticides may establish their reality.

The simultaneous use of malathion and fenitrothion would obviously hasten the selection of all above genotypes, and therefore a rapid selection of a multiresistant population would be inevitable. Thus, reliance on fenitrothion as an alternative to malathion in malaria control is dependent on the occurrence, frequency and distribution of the generalised mechanism in the population and the extent to which this or other fenitrothion resistant mechanisms have already been selected either by malathion, or other insecticides used in the environment. The fact that the possible existence of such a mechanism appears to be indicated in three species, A. culicifacies from India, A. albimanus from El Salvador and A. stephensi from Iran may suggest that this mechanism may not be uncommon, though the possibility of its being characteristic of certain species only must not be overlooked. In fact, in all the populations where the resistance spectra were

studied, while positive evidence of resistance can be considered confirmatory, negative evidence (failure to detect resistance in any population) need not necessarily mean absence of resistant factors in the species concerned. It should be emphasised that in most instances only a single population from the progeny of a limited field sample has been investigated in such studies.

Thus in any attempts to avoid or delay the development of resistance in populations, prior knowledge of the presence and frequency of the different resistant mechanisms, and the insecticides likely to select them may be of immense importance in decisions on the choice and sequence of use of insecticides to be used. It is apparent that as far as the OP's are concerned, that both malathion and fenitrothion should not be used together in populations having the genotypes just described.

Finally, as already pointed out by Davidson and Zahar (1973) the survivals from discriminating dosages while indicating the presence of resistance, do not necessarily represent the actual survivals occurring in the field situation. Therefore, when resistance is detected field studies should be implemented to assess its operational implication prior to any consideration of alternative countermeasures.

Table 21. Tolerance characteristics of the populations of *Anopheles vexans* tested to various organochlorines, organophosphates, carbamates and pyrethroid insecticides  
(S = susceptible ? = low levels of resistance R = resistant)

	<i>A. collicifacies</i>			<i>A. stephensi</i>					<i>A. albopictus</i>		<i>A. subpictus</i>	<i>A. maculipes</i>	<i>A. aegypti</i>	<i>A. hyrcanus</i>		
	CHL/SHL	CHL/PA	CHL/MD	India	SE/LY	SE/BAH	SE/NOG	SE/YS*	SE/YS*	Yunnan	El Salvador	Turkey	Greece	Greece	Greece	Turkey
chlorfenvinphos	S	S	R	S	S	-	-	S	R	S	R	R	S	R	-	R
phoxin	-	-	-	S	S	-	-	S	R	S	R	R	-	-	-	-
lufenoxin	S	S	R	S	R	-	-	R	-	S	R	R	-	R	-	R
piraliphos-methyl	S	S	R	S	S	-	-	S	R	S	R	R	S	R	-	S
fenitrothion	S	S	S	S	S	S	S	S	-	S	S	R	S	S	S	S
fenitrothion	S	S	R	S	S	?	S	S	R	S	R	R	?	R	S	R
phenothione	-	-	-	S	S	-	-	R	-	S	R	-	-	-	-	-
malathion	S	S	R	S	S	?	?	R	R	S	R	-	S	S	S	?
malathion	S	S	R	S	S	-	-	R	-	S	R	-	-	-	-	-
propoxur	S	S	S	S	S	S	S	S	-	S	R	R	?	R	?	-
permethrin	-	-	-	?	S	S	S	S	-	S	S	R	-	R	-	-
decamethrin	-	-	-	?	S	S	S	S	-	-	S	R	-	R	-	-
DPP	R	R	R	?	R	R	R	R	-	S	R	R	-	R	-	R
dieldrin	S	R	R	-	-	-	-	-	-	-	R	R	-	-	R	R

\* Laboratory selected



SUMMARY

- (1) The susceptibility status of a number of populations of seven species of anopheline vectors of malaria was compared by exposure of these to a series of organochlorines, OP's, carbamates, and some pyrethroids.
- (2) Investigations involving genetic studies, use of synergists and determination of resistance spectra were made in an attempt to characterise some of these resistances.
- (3) While a population of A. culicifacies from Sri Lanka was resistant to only DDT, that from Pakistan resisted both DDT and dieldrin. The strain from Maharashtra State, India, on the other hand showed resistance to organochlorines, as well as a number of OP compounds, including malathion and fenitrothion. The high malathion resistance was of a completely dominant nature. Two factors, the specific carboxyesterase mechanism and a more generalised mfo mechanism were suggested to be involved in this resistance, the latter imparting cross resistance to fenitrothion in addition and possibly to a number of other OP's. The DDT resistance in the population of this species from Sri Lanka was attributed mainly to a specific DDT-ase mechanism.
- (4) In A. stephensi, the presumably susceptible strain from Delhi, India, showed some tolerance to both DDT and pyrethroids, while a strain from Bangalore, India resisted DDT and iodofenphos among the OP's. In addition this population also showed the potential to develop resistance to malathion, fenitrothion, and propoxur. The laboratory selection of a malathion resistant population from Iran, in

which the specific carboxyesterase mechanism was selected, showed no cross resistance to fenitrothion. Subsequent selection of this malathion resistant population with fenitrothion however, showed a concurrent increase in tolerances to a number of OP's suggesting a relationship between these OP resistances. A potential to develop propoxur resistance has also been demonstrated. The malathion resistance in the Iranian strain was of an incompletely dominant nature, and was found to be due to a single genetic factor. The DDT resistance in both the Bangalore and the Iranian strains was primarily attributed to the DDT-ase mechanism.

(5) The population of A. albimanus from El Salvador was resistant to organochlorines, OP's (with the exception of fenthion) and the carbamates, in contrast to a highly susceptible strain from Panama. The malathion resistance in the former was attributed to a carboxy-esterase as well as an mfo mechanism.

(6) While populations of A. sacharovi from Greece were resistant to only the organochlorines, those from the Chukurova Plain of Turkey were resistant to organochlorines, OP's (including fenthion), carbamates and even pyrethroids. Malathion resistance was rare, however. The use of synergists suggested the possibilities of involvement of both hydrolytic esterases as well as mfo's in the resistances to the OP's, fenitrothion and iodofenphos. The DDT resistance was shown to be caused by DDT-ase, mfo's and a third factor, possibly a knockdown resistant mechanism and the pyrethroid resistance in this population was considered to be due to a cross-resistance imparted by one or more of these mechanisms.

(7) A. maculipennis showed a pattern of resistance somewhat comparable to that of A. sacharovi, but at a lower level.

(8) A. hyrcanus from the Chukurova Plain of Turkey also showed resistance to organochlorines, OP<sup>B</sup> (including fenthion) but was more susceptible to the carbamates.

(9) Only one population of A. superpictus was studied, from Greece, and was found to be susceptible to almost all the insecticides tested.

(10) Detailed comparisons of different multiple resistant populations are made and the possible causes for such differences are postulated.

(11) The significance of the results has been discussed in terms of their importance in future malaria control activities.

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